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# TULAREMIA

## STUDY OF RAPIDLY FATAL CASE (FOUR DAYS, SEVEN HOURS) *

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The literature affords only five reports of postmortem examinations in fatal human cases of tularemia. The purpose of the present undertaking is to record the clinical and pathologic findings in the most rapidly fatal case of tularemia of which there is any record. In the case to be described, death occurred four days and seven hours after the onset of illness.

It has been generally assumed that the fatal termination of tularemia is a rare occurrence. Recent experiences justify the belief that death is of relatively frequent occurrence. Up to May 1, 1928, 613 cases of tularemia had been recorded by Francis. Among these there have been twenty-three deaths, a mortality of 3.75 per cent. I am of the opinion that there have been many more deaths from tularemia than these figures would indicate. In the course of a study of fifty-three confirmed nonfatal cases of tularemia occurring in Dayton, Ohio, the details of forty-eight of which are published elsewhere,[1] I learned of seven deaths which were in all probability due to tularemia. Based on these statistics death has occurred in approximately 11 per cent of the cases in Dayton.

In 1924, Verbrycke [2] published the first report of the autopsy in a case in which the onset occurred on the fifth day after two market rabbits were dressed and in which death occurred eighteen days after the onset of illness. Serum collected on the sixteenth day of illness agglutinated *Bacterium tularense* in all dilutions to 1:80. Cultures of *Bacterium tularense* were obtained at the Hygienic Laboratory, at Washington, D. C., from guinea-pigs, rabbits and white mice which died with acute forms of tularemia after inoculation with splenic tissue obtained at the autopsy. In the lower lobe of the left lung and in the upper and lower lobes of the right lung, small discrete nodules about the size of a small

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* From the Pathological Laboratories of the Miami Valley Hospital.

* Presented, in part, at the annual meeting of the American Association of Pathologists and Bacteriologists, Washington, D. C., May 2, 1928.

1. Simpson, W. M.: Tularemia (Francis' Disease): A Clinical and Pathological Study of Forty-Eight Nonfatal Cases and One Rapidly Fatal Case, with Autopsy, Occurring in Dayton, Ohio, Ann. Int. Med. **1**:1007, 1928.

2. Verbrycke, J. R.: Tularemia, with Report of a Fatal Case Simulating Cholangeitis, with Postmortem Report, J. A. M. A. **82**:1577 (May 17) 1924.

shot were found distributed over the surface and throughout the parenchyma of the lungs; one nodule, however, was about the size of a walnut. Enlarged caseous peribronchial lymph nodes were noticed at the right hilum. Scattered over the surface of the spleen and throughout the pulp were many small, circumscribed, firm, yellowish white nodules, the size of millet seed. Enlarged lymph nodes were not noted elsewhere.

Microscopically, foci of active tuberculosis were found in the bronchial lymph nodes. Histologic studies of the tularemic lesions of the lungs revealed that they were most prevalent in the tissues adjacent to the bronchioles and their surrounding vessels and apparently had their origin in lymphatic structures. They showed fibroblastic proliferation together with endothelial proliferation of the smaller blood vessels, in some cases producing obliteration. The central portions of these areas were necrotic, and the whole mass was infiltrated with leukocytes, at least one half of which were lymphocytes. At the margins were irregular masses of fibroblasts, and the surrounding alveolar walls showed increased connective tissue. The splenic lesions consisted of a relatively narrow zone of fibroblasts, a rather wide zone of mononuclear cells and a small area of central necrosis. The hepatic lesions resembled those seen in the lung and spleen.

Francis and Callender [3] published the postmortem report of two other cases of tularemia in man. The first of these cases was that in which the autopsy was performed by Bruecken, pathologist at the St. Francis Hospital, Pittsburgh. The patient had dressed rabbits five months previously, at which time infection is supposed to have taken place. Serum collected two months prior to death agglutinated *Bacterium tularense* in all dilutions to 1:320. A biopsy of an axillary lymph node removed two months after the onset of illness showed several areas of necrosis surrounded by a thick wall made up of epithelioid cells interspersed with fibroblasts and infiltrated with leukocytes, of which polymorphonuclears predominated. The central mass was largely made up of necrotic cells and polymorphonuclear leukocytes. Many giant cells of the Langhans type were found in the epithelioid granulation tissue.

At the autopsy, enlarged peribronchial lymph nodes were found in the left lung. Near the capsule of the liver was a circumscribed nodule containing caseous material; at another point there was a similar nodule, chiefly fibrous. Several caseous nodules were found in the spleen. The suprarenals showed multiple opaque yellowish and grayish transluscent areas. The left axillary lymph nodes were enlarged and contained multiple yellowish foci. Cultures of heart's blood showed *Streptococcus hemolyticus.*

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3. Francis, E., and Callender, G. R.: Tularemia: The Microscopic Changes of the Lesions in Man, Arch. Path. **3**:577 (April) 1927.

Histologic examination of tissue from the axillary lymph node removed at the time of autopsy showed marked polymorphonuclear infiltrations, presumably due to the septicemia caused by *Streptococcus hemolyticus* which developed toward the end of the patient's illness. The tularemia foci were obscured by the leukocytic infiltrations. In some areas, the necrotic centers were surrounded by a relatively narrow zone of radially placed fibroblasts infiltrated with a few epithelioid cells and many polymorphonuclear leukocytes. The lesions of the bronchial lymph nodes resembled those of the axilla, except for the lack of polymorphonuclear infiltration. The lesions were essentially the same as those found in the axillary node removed prior to death. Many Langhans' giant cells were present. Special stains for reticulum and collagen revealed that reticulum was not produced in the wall of the necrotic areas but that abundant collagen fibers were formed by the connective tissue cells. A lesser omental lymph node showed one small nodule essentially similar to those already described. The hepatic and splenic lesions resembled those described for the bronchial lymph nodes. Scattered throughout the cortex of the suprarenal glands were multiple necrotic foci showing considerable leukocytic infiltration but no fibroblastic or epithelioid proliferation. The blood vessels near the tularemic lesions as seen in the tissues removed at autopsy revealed the endothelial proliferation first described by Permar and Weil.[4]

The third case was also recorded by Francis and Callender.[3] Cultures of *Bacterium tularense* were obtained from guinea-pigs inoculated with blood collected on the fourth day of illness, from pus collected from the suppurating right posterior auricular gland on the fourteenth day of illness and from splenic tissue taken at necropsy twenty-six days after the onset of illness. Secondary pneumonia of the right lung developed during the last few days of life. At necropsy, which was restricted to the examination of the abdominal viscera, the spleen was found to be studded over the entire surface and throughout the pulp with whitish nodules from 1 to 3 mm. in diameter. Gross examination of the liver did not show any abnormalities. The tissue from the liver was not examined microscopically. Microscopic studies of the splenic lesions showed miliary and submiliary necrotic foci scattered throughout the spleen. These were made up of small necrotic areas surrounded by a delicate zone of fibroblasts interspersed with numerous macrophage cells.

In April, 1928, Bardon and Berdez [5] reported their observations in a case of tularemia in which the patient died sixteen days after cutting up the carcass of a wild rabbit found dead. Serum collected eleven days

4. Permar, H. H., and Weil, G. C.: The Histopathology of the Subcutaneous Lesions in Tularemia in Man, Am. J. Path. **2**:263, 1926.

5. Bardon, R., and Berdez, G.: Tularemia: Report of a Fatal Case with Postmortem Observations, J. A. M. A. **90**:1369 (April 28) 1928.

after the onset of illness agglutinated *Bacterium tularense* completely in all dilutions to 1:160 and gave partial agglutination in dilution of 1:320. Enlarged left epitrochlear and axillary lymph glands were found. Multiple whitish nodules, measuring up to 0.4 cm. in diameter, were found scattered over the pleurae. Both lungs showed extensive bronchopneumonia. The spleen weighed 310 Gm. Beneath the capsule were many whitish nodules. On section, these nodules were found throughout the spleen. A few whitish nodules, similar to those seen in the spleen, were found in the liver. Microscopic examination showed the splenic tissue to be made up of areas of necrosis, in which a few polymorphonuclear leukocytes and chromatin detritus could be recognized. In the liver were numerous necrotic areas with marked polymorphonuclear and lymphocytic infiltrations. The lesions in the lungs involved several alveoli and bore considerable resemblance to the small areas of caseation found elsewhere. In all of the organs studied, there was a singular absence of Langhans' giant cell forms.

More recently, Goodpasture and House [6] have reported their observations in a case of the ulceroglandular type which terminated fatally fifteen days after the onset of the illness. Serum collected twelve days after the appearance of the primary lesion was found by Jones to agglutinate *Bacterium tularense* in dilutions up to 1:80. (Goodpasture and House stated that half of the serum collected on the twelfth day was sent to Francis, who reported that agglutination did not occur. The specimen actually received by Francis [7] was collected on the fifth day of illness.) On the right index finger was a circular ulcer 8 mm. in diameter, with a necrotic base. The right axillary lymph nodes were distinctly enlarged, as was a right subclavicular node. The anterior mediastinal, deep cervical, tracheobronchial, periesophageal and periaortic lymph nodes were enlarged. The lymph nodes along the lesser curvature of the stomach, the peripancreatic lymph nodes and those at the porta of the liver were likewise enlarged. The liver was enlarged and showed scattered tiny gray dots simulating miliary tubercles. The spleen was greatly enlarged (950 Gm.). Beneath the capsule and on the cut surface were

6. Goodpasture, E. W., and House, J.: The Pathologic Anatomy of Tularemia in Man, Am. J. Path. **4**:213, 1928.

7. Dr. Francis said: "After communicating with Dr. E. W. Goodpasture we find that an error in recording was made by Goodpasture and House (Am. J. Path. **4**:216 and 225 [May] 1928) in the statements that serum collected from their case, A. T. R., on the thirteenth day of illness was reported negative for agglutination of *Bacterium tularense* by me, but positive (1:80) by Dr. R. L. Jones. That sample was not submitted to me. My test was made on a previous sample, collected on the fifth day of illness of their patient and was negative—a result which I have consistently found in all of thirty-three cases of tularemia tested for antitularense agglutinins in the first week of illness."

seen multiple chalky white and yellowish opaque areas measuring up to 1 cm. in diameter. The largest spots were irregular in outline.

Histologically, the lungs did not show focal necroses. In the liver were a few acute focal lesions. The earlier ones consisted of foci of polymorphonuclear and large mononuclear cells with little necrosis in the center of the group. The larger areas showed central necrosis and caseation, with a marginal zone of epithelioid cells. Langhans' cells were not present. The splenic lesions showed necrotic, sometimes caseous centers with a marginal zone of epithelioid cells, devoid of Langhans' cells. In some areas were many polymorphonuclear cells. In the right axillary lymph nodes were circumscribed areas of necrosis with central caseation and a well developed marginal zone of epithelioid cells, without Langhans' cells. In some of the blood vessels near the caseous foci thrombi were found. Sections from the other lymph nodes showed numerous similar lesions with evidence of more recent origin in that none showed such advanced caseation. Micro-organisms could not be demonstrated in the sections. Sections through the initial lesion of the right index finger showed the base to be composed of necrotic tissue which extended quite deeply. The type of necrosis resembled that seen in the centers of lymphatic foci. There was no evidence of acute inflammation in the tissue surrounding the ulcer.

### REPORT OF RAPIDLY FATAL CASE

On the afternoon of Nov. 25, 1927, F. W., a negro, aged 25, a dresser of rabbits and chickens at a market in Dayton, was admitted to the receiving ward of the Miami Valley Hospital as an emergency case. He appeared well developed and muscular; he was acutely ill and delirious and was unable to give a clear account of his illness.

From his wife it was learned that the patient had dressed several hundred rabbits and chickens at the market since the beginning of the "rabbit season," which, in Ohio, begins on November 15. He developed multiple small blisters and fissures, together with desquamation of the epidermis of both hands, more marked on the dorsum of the right hand. His wife attributed these to the immersion of his hands in hot water while picking the feathers from chickens. He felt well until one week after he first noticed these lesions on his hands. On the morning of November 22, the patient complained on arising of a severe headache. He went to work, but about 9 a. m. he felt considerably worse and was forced to go home and go to bed. On the afternoon of this day, it was noticed that the glands of his right elbow and in the right armpit were swollen and tender. He remained in bed and continued to grow worse. On the morning of November 25, he had slight bloody expectorations. He was unable to take food; he vomited for the first time on this morning and became delirious.

In the hospital, the patient was exceedingly restless, complained bitterly of pain in his head, in his back and legs, and was unable to move himself. His temperature on admission was 106 F. The physical examination, except for the external examination, gave essentially negative results. The skin over the entire right hand from the finger tips to the wrist showed extensive exfoliation with many deep irregular fissures extending into the corium. There was no well defined ulcer. The skin of the fingers of the left hand showed a much less marked desquamation.

The right epitrochlear lymph glands were moderately enlarged, the average size being that of a kidney bean. These were tender to palpation. The right axillary nodes were enlarged to the size of a small orange. These were exquisitely painful and caused the patient to cry out whenever they were touched. The lungs showed slight impairment of resonance below the fourth right rib, with bronchial breathing and fine crepitant râles. The heart revealed nothing except tachycardia.

At that time it was thought that the patient had right lobar pneumonia with septicemia. In view of the significant occupational history and the presence of the ascending lymphangitis and the epitrochlear and axillary adenitis, it occurred to the intern, Dr. H. W. Harris, who examined the patient when admitted, that the diagnosis might be tularemia. The overwhelming character of the infection seemed to be the only argument against such a diagnosis. I was called in consultation and concurred in the diagnosis of tularemia. Ten cubic centimeters of blood was withdrawn at this time for inoculation into guinea-pigs and agglutination tests. One cubic centimeter of serum was immediately injected into each of two guinea-pigs. The remainder was sent to Dr. Edward Francis, at the Hygienic Laboratory, Washington, D. C.

All attempts to reduce the patient's temperature were of no avail. On the morning of the 26th, his condition was obviously worse. He grew more delirious, and it became necessary to employ mechanical restraint. He developed urinary and fecal incontinence, associated with a profuse watery diarrhea; his speech was thick and incoherent, and he could be aroused only with great difficulty. The temperature remained at 106 F. until 4:23 p. m., when the patient died, just twenty-four hours after his admission and four days and seven hours after the onset of his illness.

A blood count, made on the morning of the 25th, showed 5,200,000 red blood cells; hemoglobin content, 90 per cent; white blood cells, 15,400; differential count: polymorphonuclear neutrophils, 91 per cent and small lymphocytes, 9 per cent.

There was some difficulty encountered in obtaining permission for postmortem examination, and it was impossible to begin the autopsy until 10 a. m. on November 27.

*Pathologic Anatomy.*—The body was slender, well developed, muscular and 162 cm. long. The general nutrition was good. The head was symmetrical and did not show scars or wounds. The facies was symmetrical, with prominent bony landmarks. The eyes showed the pupils to be moderately dilated, round, equal and regular; the sclerae were clear. A few palpable lymph nodes the size of kidney beans were found in the lower half of the anterior cervical chain. The thorax was symmetrical; the intercostal angle was less than a right angle. The abdomen was level with the margin of the ribs. The back showed well marked hypostasis over the dependent dorsal areas. The skin was fine, soft, relatively inelastic and deeply melanotic. The skin over the right hand from the finger tips to the wrist showed multiple small blisters with extensive exfoliation of the epidermis, together with many irregular fissures extending deeply into the corium. There was no distinct, sharply "punched out" ulcer. The skin of the fingers of the left hand showed a similar but less marked desquamation. The teeth were carious and showed moderate pyorrhea. The mucous membranes were moderately cyanotic. The muscles showed excellent development. Rigor mortis was present over the abdomen and lower extremities. The panniculus was practically absent. Edema was not present, and the body heat was absent. The mouth did not show ulceration. Examination of the nose did not reveal any perforation. Discharge from the ears was not present. There were no genital scars. Examination of the anus did not reveal hemorrhoids or fissures.

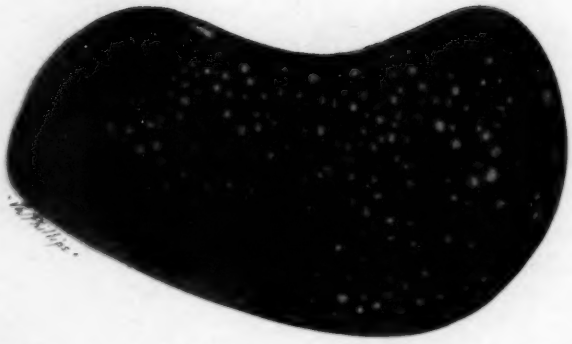

Fig. 1.—Spleen of guinea-pig which died four days after inoculation with cultures of *Bacterium tularense*.

Fig. 2.—Liver of guinea-pig which died four days after inoculation with cultures of *Bacterium tularense*.

The spinal cord and brain were not examined because of stated restrictions.

Main Incision: The panniculus was represented by a few scattered lobules of fat. The musculature was unusually well developed. The abdominal recti, especially below the umbilicus, showed a striking picture of Zenker's necrosis, presenting the typical waxy, hyaline, fish fleshlike appearance. The abdominal cavity did not contain free gas or fluid. The omentum was loosely gathered about the transverse colon and did not show adhesions. The liver descended one finger-breadth below the margin of the ribs in the right midclavicular line. The inferior pole of the spleen did not descend below the left margin of the ribs. The dome of the diaphragm was on the level with the fourth intercostal space on the right and on the level with the fifth interspace on the left. The costal cartilages were elastic and cut with ease. The cortex of the sternum was of normal thickness, and the medulla contained abundant red-brown marrow.

Thorax: The thoracic cavity did not contain free gas or fluid. The anterior borders of the lungs were two fingerbreadths apart in the anterior mediastinum in which were a few small lymph nodes which did not appear abnormal on section. The thymic outline was simulated by a thin sheet of fat, in which could be seen a few slender whitish strands of persistent thymic tissue. The pericardium was smooth, moist and shining and showed multiple healed "soldier spots" over the anterior wall of the right ventricle. Just above the apex, anteriorly, the pericardial surfaces were firmly adherent over an area about the size of a silver dollar. The pericardial sac contained about 1 tablespoonful of clear yellowish fluid. The heart was about the size of the right fist. The cardiac apex was in the fifth interspace in the left midclavicular line. The apex was sharp and was formed entirely by the left ventricle. The right border of the heart was at the right parasternal line. The wall of the left ventricle averaged 14 mm. in diameter and did not show fibrosis or infarction. The endocardium was pale, yellowish-gray and showed diffuse subendocardial fatty degenerative infiltration ("tiger heart"). Endocardial thrombosis was not present. The mitral orifice admitted two fingers with ease. The valve flaps did not show vegetations or fenestrations. The aortic ring admitted the thumb with ease. The semilunar valves did not show vegetations. The right ventricular wall averaged 6 mm. in thickness and did not show fibrosis or infarction. There was the same diffuse subendocardial fatty degenerative infiltration as in the left side of the heart. The tricuspid orifice admitted four fingers. Tricuspid valvular abnormalities were not found. The pulmonic ring admitted two fingers readily; the valves did not show vegetations. The coronary vessels were thin-walled throughout. There were multiple old firm pleural adhesions on the left lung. At the apex was a patch of healed fibroid tuberculosis about the size of a silver dollar. Multiple healed calcified subpleural tubercles were found. On section, there was marked acute passive congestion, moderate edema and no evidence of pneumonia or active tuberculosis. The right lung was similar to the left except that in the lower lobe, in the costophrenic thin margin, was a firm, reddish-brown area the size of a walnut which showed a caseous core the size of a cherry, surrounded by a thin zone of purulent infiltration. In the anterior part of the middle lobe just beneath the pleura was a similar area the size of a cherry. The bronchi contained a moderate amount of frothy edema but not any purulent exudate. The bronchial glands were hyperplastic; on an average, they were the size of a hazel-nut and contained multiple healed and caseous tubercles. The pulmonary vessels were thin-walled and elastic and did not show thrombi or emboli. The aorta was hypoplastic, thin and elastic and did not show atherosclerosis or evidence of syphilis.

Abdomen: The peritoneal surfaces were smooth, moist and shining throughout. The peritoneal cavity did not contain free gas or fluid. The spleen was about one and a half times its normal size, weighed 165 Gm. and measured 15 by 9 by 5 cm. The splenic capsule was smooth and shining. Beneath the capsule were seen multiple whitish and grayish-white areas, usually spherical but occasionally irregular, varying in size from that of a pinhead to that of a small pea. On section, similar areas were found scattered throughout the splenic pulp. The pulp was soft and deep reddish-purple. The malpighian corpuscles were not visible. The large intestine showed marked congestion of the mucosa. The appendix was in part retrocecal and did not show inflammation. The small intestine showed marked congestion of the mucosa; there was no ulceration and no hyperplasia of Peyer's patches. The duodenum contained a small amount of bile-stained mucin, and the mucosa did not show ulcers or scars. The bile passages

Fig. 3.—Section of spleen from rapidly fatal case in human being showing dissemination of focal necroses throughout the spleen. Approximately one-half natural size.

were widely patent. The stomach was slightly distended with gas and a small quantity of turbid fluid and showed congestion and increased mucin on the mucosa, but no ulcers or scars. The pancreas was of normal size and consistence. The peripancreatic lymph nodes were not hyperplastic. The liver was of normal size and weighed 1,460 Gm. The capsule was smooth and shining. On section, there was marked acute congestion and cloudy swelling, but the most careful scrutiny failed to reveal the presence of any whitish focal necroses. The wall of the gallbladder was thin; the mucosa was intact and the lumen did not contain bile sand or calculi. The portal vein did not show thrombosis. The mesenteric lymph nodes were slightly enlarged but did not have any areas of necrosis. The suprarenals were moderately hypoplastic, particularly in the medullary portion. The

kidneys were of normal size; the capsules stripped readily, leaving smooth, deeply congested, surfaces; there was marked cloudy swelling and acute passive congestion. The renal pelves and ureters were thin-walled. The abdominal aorta was similar to the thoracic aorta.

Pelvis: The penis did not show any scars. Edema of the scrotum was not present. The testes were soft and did not show any evidence of orchitis. The rectum showed marked passive congestion and no ulceration. The prostate gland was of normal size and consistence, and glandular hyperplasia was not present. The seminal vesicles were thin-walled and moderately distended with turbid seminal fluid. The urethra did not show strictures. The bladder was greatly distended with urine; the fundus was four fingerbreadths above the symphysis; the mucosa showed marked congestion in the region of the trigone.

Lymph glands: The right epitrochlear lymph glands were moderately enlarged, three of these nodes being of the size of a kidney bean. The right axillary lymph

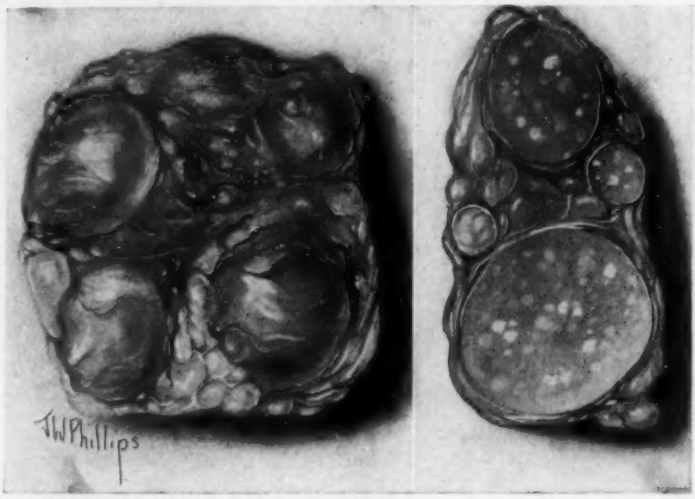


Fig. 4.—Enlarged caseous axillary lymph nodes from rapidly fatal case of tularemia. Natural size.

nodes were tremendously enlarged, many being the size of plums. These nodes were soft and friable and showed multiple whitish and grayish areas of caseous necrosis.

*Histologic Examination.*—Axillary and Epitrochlear Lymph Nodes: Scattered throughout the lymph nodes were multiple recent focal necroses, miliary and submiliary in size. As many as from three to six foci of necrosis were frequently seen in one low power field. The recentness of the necrosis was shown by the karyoschisis and karyorrhexis of the nuclei of the lymphocytes in the areas of necrosis. It was essentially an early caseation necrosis. The periphery of the necrotic areas was frequently irregular, but quite sharply defined from the contiguous more or less intact leukocytes. There was a narrow zone of early epitheliod and fibroblastic proliferation with marked hyperplasia of the reticuloendotheliocytes. The endothelial cells lining the lymph sinuses showed marked hyperplasia.

The epithelioid granulation tissue was quite devoid of new formed capillaries. Distinct foreign-body giant cells of the Langhans' type were not to be seen. Some of the histocytes appeared to have fused, forming a kind of symplasm which may represent an early stage in the formation of the Langhans' giant cells. A few scattered polymorphonuclear leukocytes were found at the periphery of some of the larger foci of necrosis. The greater part of the normal lymph node structure was destroyed. Distinct germ centers were not found. The blood capillaries were widely dilated and engorged with red blood cells. The small blood vessels did not show proliferation of the endothelial cells. Paraffin sections fixed in alcohol did not show tubercle bacilli by the Ziehl-Neelsen method as applied to

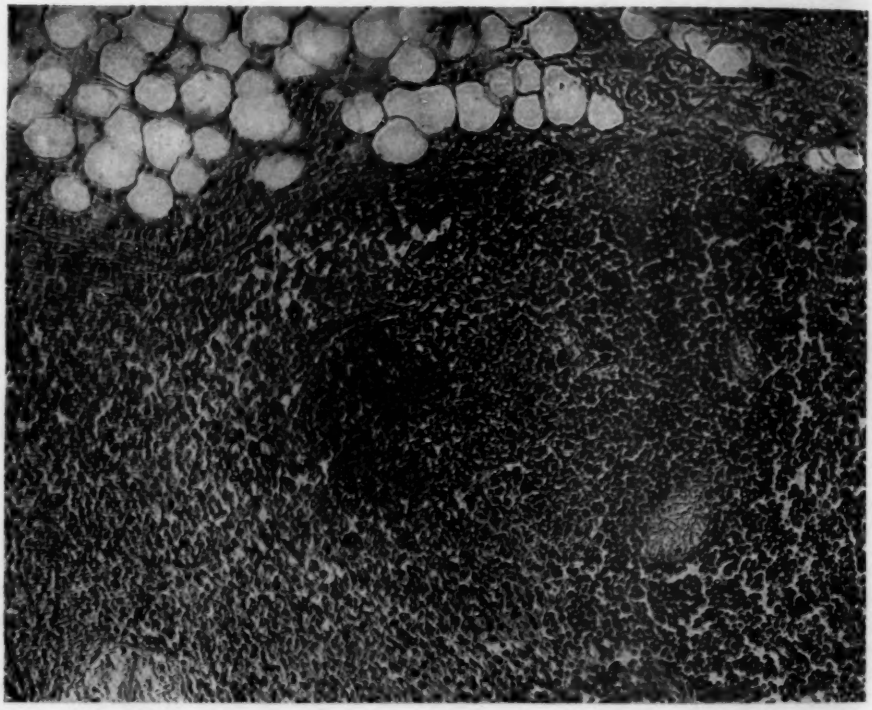

Fig. 5.—Submiliary focal necrosis of axillary lymph node, showing early epithelioid and fibroblastic granulation tissue reaction at periphery of central area of caseous necrosis. The inflammatory reaction extends into the surrounding axillary fat.

sections. Smears made from the nodes before fixation did not show acid-fast organisms.

Cervical Lymph Nodes: The cervical lymph nodes showed acute lymphadenitis with wide dilatation of the lymph sinuses and hyperplasia of the endothelial cells lining the sinuses, but no areas of necroses.

Bronchial Lymph Nodes: The bronchial lymph nodes showed multiple healed fibroid and caseous tubercles, but no recent foci of necrosis.

Heart: There was marked diffuse subendocardial fatty degenerative infiltration ("tiger heart"). Over the left ventricle were localized areas of healed epicarditis.

Lungs: Extreme acute passive congestion with moderate edema was present. In the right lung were two localized areas of recent caseous necrosis, with marked polymorphonuclear infiltration at the periphery. The character of the necrosis simulated the tularemic lesions in the lymph nodes. There were multiple healed fibroid and calcified tubercles with relatively inactive chronic adhesive pleuritis.

Spleen: The recent focal necroses here varied in size from that of a pinpoint to that of a small pea. The lesions were essentially similar to those found in the right axillary nodes, except that the diffuse reticulo-endothelial hyperplasia was much more marked. The necroses involved by far the greater part of practically every field. Some of the focal necroses were surrounded by a narrow zone

Fig. 6.—Axillary lymph node, showing rather extensive early caseous necrosis with peripheral zone of epithelioid and fibroblastic granulation tissue, devoid of Langhans' giant cells, with marked reticulo-endothelial hyperplasia.

of fibroblastic and epithelioid cells, while others showed little if any peripheral granulation tissue reaction. Mature Langhans' giant cells were not found. The endotheliocytes and macrophages of the lymph sinuses showed a marked degree of hyperplasia. The smaller blood vessels did not show endothelial proliferation or any obliterative changes. The malpighian corpuscles showed a decided diminution in the number of lymphocytes. The blood capillaries were widely dilated and engorged with blood.

Liver: A few scattered submiliary focal necroses, differing remarkably from the lymph node and splenic lesions in that there was little if any caseation, were present. Localized areas of liver cells were replaced by areas of rather extensive

reticulo-endothelial proliferation. There was little evidence of fragmentation of the chromatin of the liver cells. Many of the hepatic lesions were exceedingly small, in some instances replacing approximately from six to twelve of the liver cells. There was no constant lobular distribution, some of the lesions appearing near the islands of Glisson, others near the central vein and still others in the intermediate lobular zone.

Stomach: On the mucosa was an excess of mucin; marked acute passive congestion of the wall was present.

Kidneys: The kidneys showed a remarkable microscopic picture, in that the degenerative changes were rather sharply restricted to only one part of the tubular system. The nuclei of the epithelium of practically every one of the proximal convoluted tubules showed karyorrhexis, while the nuclei of the epithelium of the distal convoluted tubules and collecting tubules, together with those of the

Fig. 7.—Spleen, showing the multiplicity of the foci of necrosis and the diminution in the number of lymphocytes of the malpighian corpuscles. Low power magnification.

glomeruli, were preserved. All of the renal epithelial cells showed marked cloudy swelling. In most of the distal convoluted tubules were hyaline casts. In the collecting tubules were multiple large granular and hyaline casts. There were no focal necroses.

Suprarenals: The hypoplasia of the medulla was marked. The cortex showed patchy lipoidosis. Focal necroses were not seen.

Striated Muscle (rectus abdominis): The fibers were greatly swollen and the cytoplasm hyalinized. The nuclei were destroyed. The myofibrils showed fragmentation, and there was a marked difference in the eosinophilic staining qualities of the cytoplasm. The areas of Cohnheim were indistinct. The microscopic picture was that of typical intracellular coagulation necrosis of the Zenker type.

*Anatomic Diagnosis.*—The condition was diagnosed as tularemia: multiple fissures and exfoliating dermatitis of the hands, most marked on right; ascending lymphangitis of right upper extremity, with epitrochlear and axillary lymphadenitis; multiple recent focal necroses of the epitrochlear and axillary nodes, spleen, liver and right lung; marked cloudy swelling and localized simple necrosis of the liver and kidneys; subendocardial fatty degenerative infiltration; healed tuberculosis of the lungs and bronchial nodes; terminal right-sided cardiac dilatation with relative tricuspid and pulmonary insufficiency; pulmonary congestion and edema; old adhesive pleuritis and pericarditis; marked Zenker's necrosis of recti abdominis.

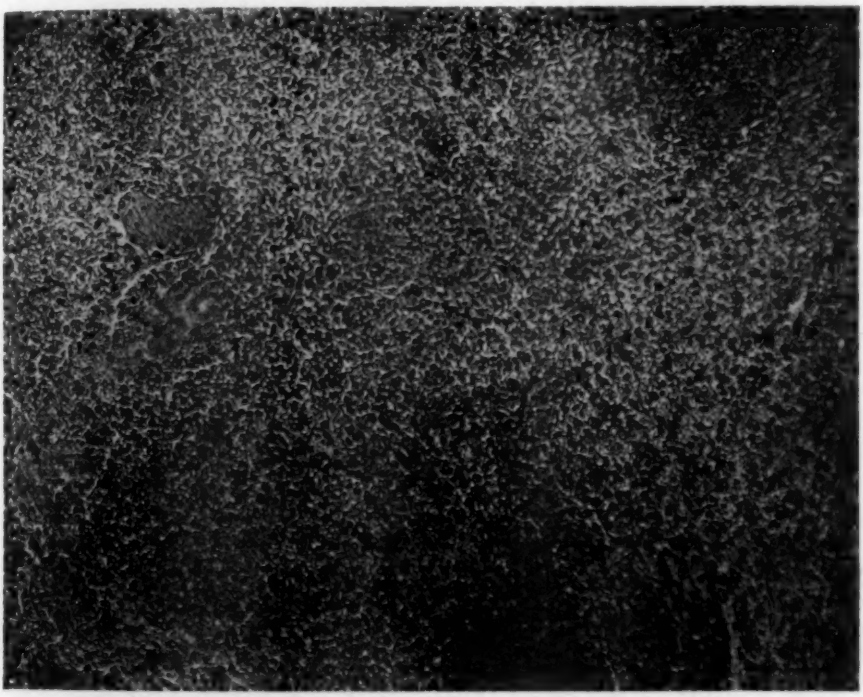

Fig. 8.—Spleen, showing small focus of early necrosis with marked diffuse reticulo-endothelial hyperplasia.

*Serologic and Bacteriologic Examinations.*—Small pieces of a right axillary lymph node were rubbed up in a sterile mortar, suspended in physiologic sodium chloride solution and strained through coarse gauze. Two healthy guinea-pigs were inoculated subcutaneously in the anterior abdominal wall.

The first of the two guinea-pigs inoculated with the patient's serum collected on the day before death (November 25) died on November 30, and autopsy was performed immediately. The inguinal lymph nodes were greatly enlarged and showed extensive caseation. The characteristic "spotted spleen and liver" were found. A drop of heart's blood was placed on each of twelve tubes containing cystine dextrose peptone-meat infusion agar (Francis' medium). In four of the

culture tubes, the medium was enriched with 5 per cent human serum. Tubes of nutrient agar and broth were inoculated at the same time. The second of the two guinea-pigs inoculated on November 25 died during the night of November 30, and December 1, and the procedures already outlined were carried out.

Both of the guinea-pigs inoculated with the suspensions of fragments of axillary lymph node tissue immediately after the autopsy died during the night of November 30 and on December 1 and showed the same grayish granular caseation of the enlarged lymph nodes of the groin and great numbers of small whitish foci of necrosis in the spleen and liver. Tubes containing Francis' medium were inoculated directly with heart's blood and fragments of splenic tissue.

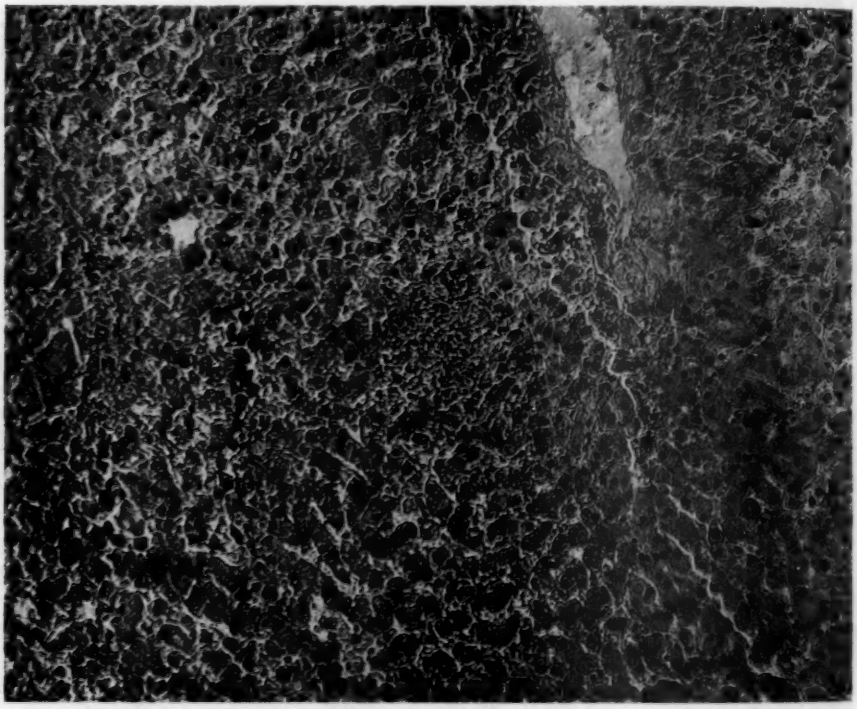

Fig. 9.—Liver, showing small area of necrosis, with liver cells replaced by reticulo-endothelial hyperplasia. Marked cloudy swelling of liver cells.

On the afternoon of November 30, a telegram was received from Dr. Francis which stated that the serum was negative for tularemia because it was taken too early in the disease. He advised that tuberculosis be excluded. The negative serologic report from Dr. Francis was anticipated because of the fact that the blood had been taken on the third day of illness and in the presence of an overwhelming bacteremia. It is a well known fact that agglutinins do not develop during the first week of the disease. The serum was sent to Dr. Francis with the hope that he might succeed in recovering the organism.

The tubes containing cystine agar medium, inoculated on November 30, showed the first growth of the characteristic grayish-white small circular colonies on December 3. The growth on the cystine agar which had been enriched with

5 per cent human serum was more luxuriant than that on the other cystine agar mediums. Sterile salt solution suspensions of the organism were injected subcutaneously and intraperitoneally into three healthy guinea-pigs. The animals thus inoculated died in four days (December 7), with the characteristic tularemic lesions, and the organism was again recovered on our human serum modification of Francis' medium from the heart's blood and splenic tissue. The organism was then passed through twelve guinea-pigs, four series with three guinea-pigs in each series, and the organism successfully recovered in each instance. The organisms recovered from the animals were taken off in 0.2 per cent solution of formaldehyde and used as an antigen for agglutination against known antitularense serum. In

Fig. 10.—Liver, showing extensive reticulo-endothelial hyperplasia, with little caseous necrosis. High power magnification.

each instance the organisms agglutinated promptly to the full titer of the known serum (1:1,280).

The hair was shaved from the abdomen of four healthy guinea-pigs, and the abraded skin was rubbed gently with fragments of splenic tissue from animals recently dead of tularemia. Three of the guinea-pigs died within four days, and one succumbed on the fifth day. The autopsy revealed the characteristic gross lesions, and in each instance the organism was recovered from the heart's blood or splenic tissue of the dead animal.

In order to determine whether or not the organism thus recovered would penetrate unbroken skin, fragments of splenic tissue were rubbed gently on the

unshaved, unabraded intact skin of the abdomen of four guinea-pigs. Two of the animals died, one on the fifth day and one on the sixth day, with the characteristic gross and microscopic lesions; the organism was recovered from both.

Tiny fragments of splenic tissue removed from animals recently dead of tularemia were placed in the right conjunctival sac of two healthy guinea-pigs. On the following day, both animals showed excessive lacrimation. On the third day a grayish-white thin film developed over the bulbar conjunctiva, with marked capillary injection and multiple minute papules of both the bulbar and the palpebral conjunctiva. Both animals died on the fifth day with caseous regional lymph nodes and the characteristic spotted spleen and liver, and from both animals the organism was successfully recovered.

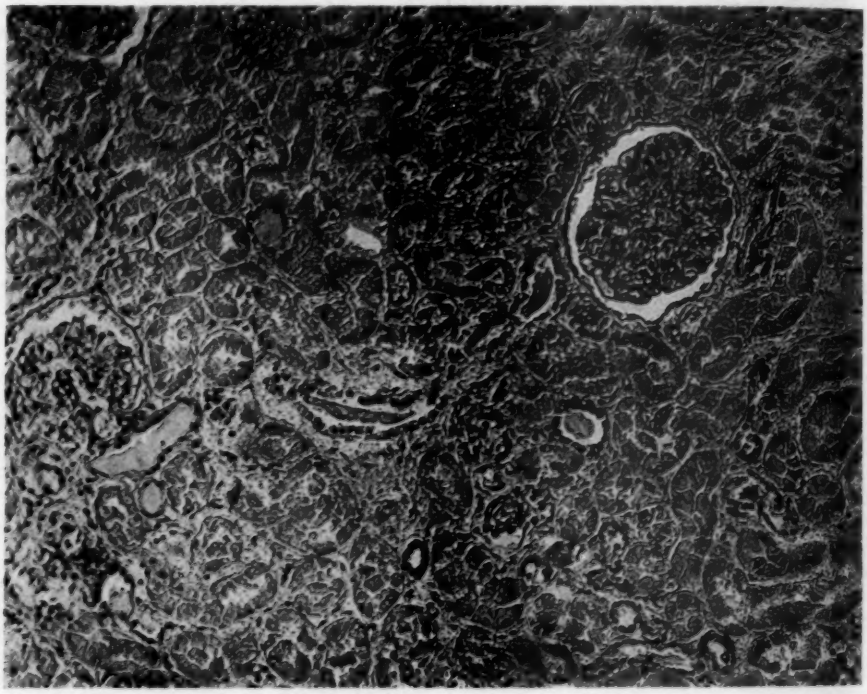

Fig. 11.—Cortex of kidney, showing intact glomeruli, simple necrosis of proximal convoluted tubules, and intact nuclei of distal convoluted tubules, nearly all of which contain hyaline tubular casts.

On Dec. 20, 1927, two dogs, two guinea-pigs and two Belgian hares were inoculated with virulent cultures of *Bacterium tularense*. The suspensions from several culture tubes were mixed together, and all six of the animals were inoculated with the same material. The two guinea-pigs succumbed in four days, with the characteristic gross and microscopic manifestations of tularemia, and the organism was recovered in pure culture. One of the Belgian hares died in seven days, the other in eight days; postmortem examination showed the characteristic lesions of tularemia, and the organism was recovered from the heart's blood and splenic tissue. The two dogs remained well. Both animals were killed on

Feb. 20, 1928, two months after inoculation, and complete autopsies with careful microscopic studies did not reveal any evidence of tularemia. Apparently the dog possesses a natural immunity to the disease. Francis [8] found that the silver fox, likewise, possesses a natural immunity.

Because of the fact that twenty skilled laboratorians have succumbed to this disease during the course of their investigations as the result of making post-mortem examinations on infected animals or as the result of handling infected wood ticks, unusual precautions were employed throughout all of the experimental work. The dead animals were immersed in 10 per cent solution of formaldehyde for five minutes before each autopsy. Sterile gowns, sterile gloves, gauze masks and glasses were worn by the prosector. All tissues were handled with sterile

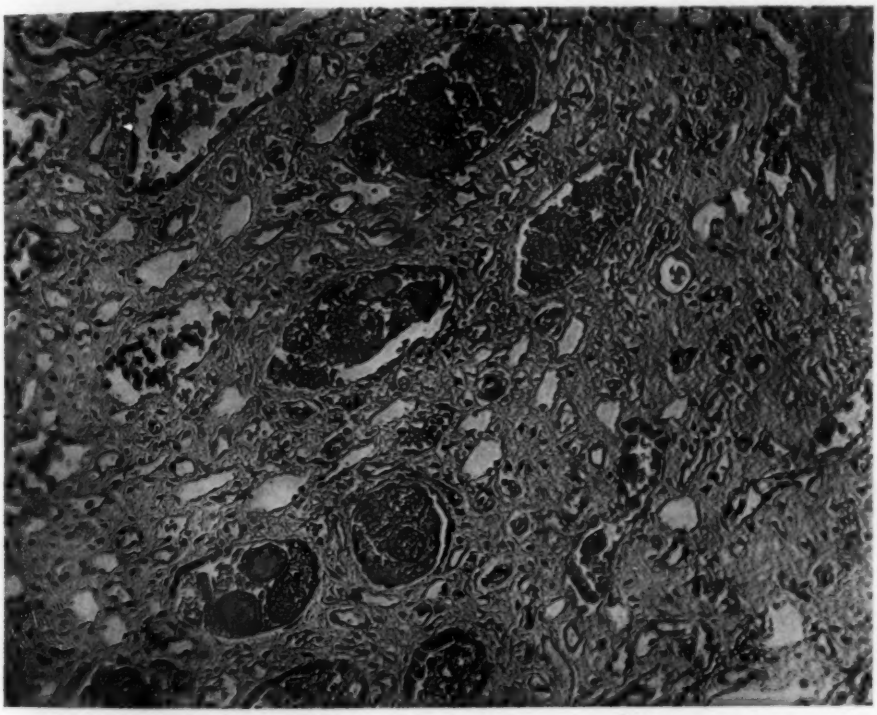

Fig. 12.—Medulla of kidney, showing large hyaline and granular tubular casts in collecting tubules.

instruments. All of the materials used were sterilized immediately after the autopsy, and the benches on which the work had been carried out were washed off with 10 per cent solution of formaldehyde and running water. The prosector's hands were washed with soap and water, then immersed in 10 per cent solution of formaldehyde for a few minutes and then held under running water. The cages in which the animals died were immersed in boiling water. In order to limit the possibilities for the development of the infection, I did all of the experimental work. No person in the laboratory contracted the disease.

8. Francis, E.: Personal communication to the author.

On December 13, the following report was received from Dr. Francis:

"Serum of F. W., on which I reported negative agglutination, November 30, was injected, November 29, subcutaneously into pig no. 1 and intraperitoneally into pig no. 2. Pig no. 1 was killed while dying, December 5, and showed the typical lesions of tularemia—caseous lymph nodes and spotted spleen and liver. Pig no. 2 died December 2, not showing gross lesions of the lymph nodes, but showing the spotted spleen and liver.

"Cultures of blood from the heart of pig no. 2, taken at the moment of death, showed growth, December 4, from which a transfer was made, December 5. The latter was taken off in 0.2 per cent solution of formaldehyde and used December 11 as an antigen for agglutination against a known antitularense serum, a known

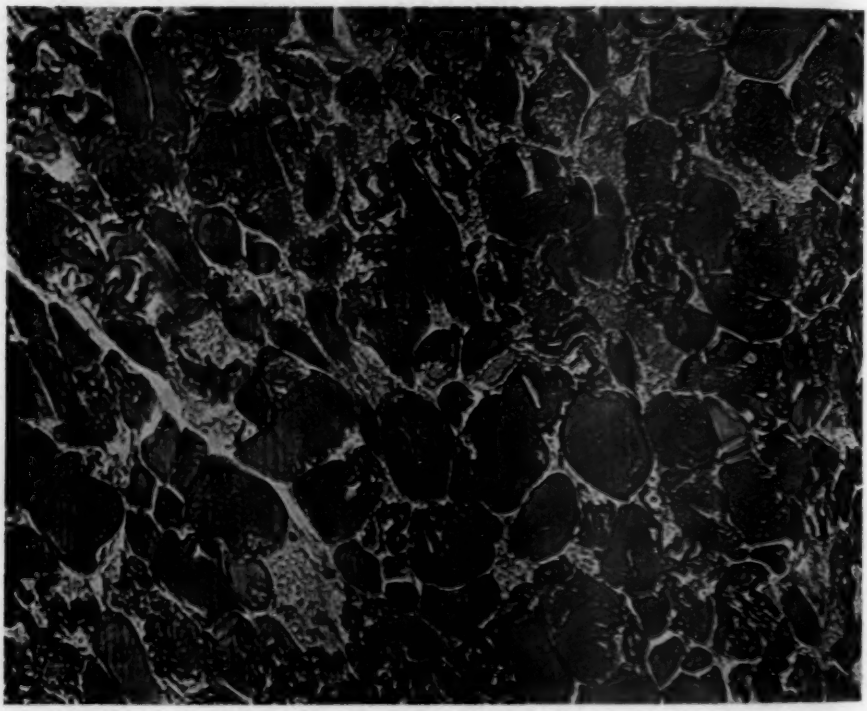

Fig. 13.—Striped muscle (rectus abdominis), showing large swollen hyalinized fibers, with necrosis of peripheral nuclei. Typical Zenker's necrosis.

tularense organism being also set up in the agglutination test. Both organisms agglutinated out promptly to the full (1:1,280) titer of the serum.

"Transfers from the spleen of pig no. 2 to the shaved abraded skin of the abdomen of two pigs caused their death, December 6, and they showed the typical lesions of tularemia—caseous lymph nodes and spotted spleen and liver.

"Your case is the most rapidly fatal (five days) of any on record. . . . I admire the way you have attacked the situation, but I must warn you that if you continue to perform autopsies on infected pigs you are sure to go to the hospital—there have been twenty laboratory infections of man in five laboratories in this country, in England and in Japan."

COMMENT

The case reported is unquestionably the most rapidly fatal case of tularemia on record. Death occurred four days and seven hours from the time of onset of illness. It offered an unusual opportunity to study the earliest histologic manifestations of this disease. In the other five fatal human cases which came to autopsy, death occurred on the fifteenth, sixteenth, eighteenth and twenty-third days and five months after the onset of symptoms.

This case possesses further interest in that death resulted from tularemia per se. All of the symptoms indicated an overwhelming bacteriemia in which *Bacterium tularense* alone was the causative organism. In most of the other reported fatalities some complicating factor existed, such as pneumonia, streptococcus septicemia, peritonitis or heart disease.

The early histologic lesions which were found simulated to a remarkable degree those found in rodents which have succumbed to the infection, either in nature or after laboratory inoculation. The most characteristic lesions were found in those lymph nodes (epitrochlear and axillary) which drained the site of primary inoculation and in the spleen. The characteristic lesions of tularemia were foci of necrosis, essentially of the caseous type, usually miliary and submiliary in size in the most recent lesions. At the periphery of most of the areas of caseous necrosis there was found a narrow zone of epithelioid and fibroblastic proliferation, with marked hyperplasia of the contiguous reticuloendothelial cells. Many of the earlier lesions did not show epithelioid granulation tissue. In the lymph nodes and spleen, the endothelial cells lining the lymph sinuses showed marked hyperplasia. The epithelioid granulation tissue was relatively avascular. In this tissue, giant cells of the Langhans' type were not found. Bardon and Berdez [4] and Goodpasture and House [5] also noted the same singular absence of Langhans' giant cells in tissues removed at autopsy. In the lymph node and splenic lesions, I was unable to find any evidence of endothelial proliferation or thrombosis of the small blood vessels such as have been reported by other investigators. In both the lymph nodes and the spleen, the normal architecture was practically destroyed by the multiplicity of the focal necroses. In the lymph nodes germ centers did not remain, while in the spleen the number of lymphocytes of the malpighian corpuscles was greatly diminished.

The regional lymph nodes reached a large size with remarkable rapidity. Three days after the onset of the illness the axillary adenopathy had reached the size of a small orange. Many of the individual axillary lymph nodes were the size of a cherry or a plum. The spleen, however, was only about one and a half times the normal size.

Gross examination of the liver did not reveal any small whitish foci of necrosis, but on microscopic examination a few scattered submiliary lesions were found. These differed from the lymph node and splenic lesions in the relative absence of caseous necrosis. Localized areas of the liver cells had undergone simple necrosis and were replaced by reticulo-endothelial cells.

The two lesions in the right lung were unquestionably tularemic focal necroses. The pulmonary lesions simulated those seen in the lymph nodes and spleen except that surrounding the central caseous necrosis was a zone of polymorphonuclear infiltration. In many of the fatal cases reported, without autopsies, death was said to be due to bronchopneumonia. I am of the opinion that the physical signs in such cases might well have been produced by multiple tularemic necroses. These lesions are not necessarily associated with the bronchi, since they result from the hematogenous dissemination of the process. It is erroneous to consider such foci as areas of bronchopneumonia.

The most marked manifestations of tularemia occur in the lymphoid apparatus. The sporotrichosis-like nodular lymphangitis, which occasionally develops, the regional lymphadenopathy and the splenic and hepatic lesions constitute the most characteristic findings in the disease. The reticulo-endothelial hyperplasia was one of the outstanding characteristics of the disease in this case.

Unfortunately, it was impossible to study the characteristics of the primary lesion because this case was one of the glandular type in which a demonstrable primary lesion was not present. Netherton[9] first accurately described the granulomatous character of the primary lesion.

In most instances it is difficult to distinguish the lesions of tularemia from tuberculosis. The character and course of the disease are such, however, as to leave little doubt as to their proper interpretation.

There are two recorded cases[10] in which *Bacterium tularense* was recovered from human tissues removed at postmortem examination. I found that the organism grew most luxuriantly on a human serum modification of Francis' cystine agar medium. This medium was prepared in the following manner: A stock medium was prepared consisting of beef infusion containing 1 per cent peptone, agar, 1.5 per cent, and sodium chloride, 0.5 per cent, adjusted to a $p_H$ of 7.3. When needed there was added to the stock medium 0.1 per cent cystine and 1 per cent dextrose. This was placed for fifteen minutes in an Arnold sterilizer at the temperature of flowing steam to melt the agar and sterilize the

9. Netherton, E. W.: Tularemia, with Reference to Its Cutaneous Manifestations, Arch. Derm. & Syph. **16**:170 (Aug.) 1927.

10. Footnotes 2 and 3.

cystine. It was then cooled to 45 or 50 C. and 5 per cent sterile human serum was added. It was then tubed, slanted and incubated for twenty-four hours to insure sterility.

## SUMMARY AND CONCLUSIONS

1. The increasing importance of tularemia as a disease of man is manifested by the fact that fifty-three proved cases were found in one city (Dayton, Ohio) during a period of six months.

2. That death from tularemia probably occurs more frequently than the figures gathered from the literature might indicate (3.75 per cent) is shown by the discovery of seven deaths probably due to tularemia in Dayton.

3. Five reports of postmortem examination in human cases of tularemia have appeared in the literature, death occurring after fifteen, sixteen, eighteen and twenty-six days and five months, respectively. The essential gross and microscopic observations in these cases are presented in this paper.

4. Postmortem examination in the most rapidly fatal case of tularemia in man on record (four days and seven hours) provided an opportunity to study the early gross and microscopic manifestations of the disease.

5. The characteristic "spotted spleen" and enlarged caseous regional lymph nodes were found. Careful scrutiny of the liver did not reveal gross evidence of focal necrosis.

6. Histologic studies showed the focal necrosis of the spleen and epitrochlear and axillary lymph nodes to be of exceedingly recent origin, with a narrow zone of relatively avascular epithelioid granulation tissue at the periphery of the foci of early caseous necrosis, which was devoid of Langhans' giant cell forms. Marked reticulo-endothelial hyperplasia was present. A few scattered submiliary lesions were found in the liver, which differed remarkably from those of the spleen and lymph nodes, in the relative absence of caseous necrosis. Two focal necroses were found in the right lung, each surrounded by a broad zone of polymorphonuclear leukocytic infiltration. The microscopic lesions were strikingly similar to those seen in experimental rodents which have died with acute forms of the disease.

7. The lesions of tularemia should be classed with the infective granulomas.

8. Both Francis and I recovered *Bacterium tularense* from serum collected twenty-four hours prior to death. The organism was likewise recovered from tissue from the axillary lymph nodes removed at autopsy.

The best growth was obtained on a human serum modification of Francis' cystine agar medium.

9. It was demonstrated experimentally that the organism will penetrate the unbroken skin of guinea-pigs. The oculoglandular form of the disease was produced experimentally in guinea-pigs. The dog possesses a natural immunity.

# THE HISTOGENETIC INTERPRETATION OF CERTAIN CARCINOIDS OF THE SMALL INTESTINES

## A NEOPLASM-LIKE MALFORMATION OF THE TISSUE OF THE PANCREAS *

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Carcinoids in the appendix, jejunum and duodenum are of interest on account of their relation to tumor processes.

There are two opinions as to the nature of carcinoids; first, that they represent true tumors which originate from epithelial elements of the adult mucosa of the gastro-intestinal tract; and, second, that they represent epithelial islets formed in the mucous membranes by a process of faulty ontogenesis (a dysontogenetic heterotopy), and that they may become the starting point of a neoplastic process.

A case recently studied in the New York Hospital appears to throw some light on the question. In the following, after a brief summary of the clinical history, the gross anatomic and microscopic observations will be given and a histogenetic interpretation of these observations will be attempted.

### REPORT OF A CASE

*History.*—A gastro-enterostomy for ulcer of the duodenum was performed on an Italian, aged 41, eleven years ago. After an interval of nine and a half years, the symptoms of ulcer recurred. Two days before his admission to the hospital, the patient noticed that his stools were tarry. On the day of admission, he noted a sudden sharp pain in the left part of the epigastrium, radiating to the left shoulder. Immediate laparotomy revealed an abscess in the gastrocolic ligament, which was connected with a perforation at the jejunal side of the gastro-enterostomy. The gastro-enterostomy scar was excised, the stomach and jejunum were sutured, and a jejunostomy was made. The patient died from a post-operative ileus.

*Autopsy.*—A partial autopsy revealed, in addition to the aforesaid operations, an atrophic gastritis; old peritoneal adhesions in the epigastrium; acute splenitis, and an absence of the appendix (operative). Near the upper pole of the left kidney, a solitary cyst was found, measuring 2.5 cm. in diameter, which contained many medium-sized, facetted stones. These stones resembled the typical facetted gallstones; they did not, however, contain cholesterol.

Bearing on the matter under consideration, it was noted that the mucosa of the first two portions of the duodenum contained a few irregularly arranged, whitish nodules, measuring from a few millimeters to one centimeter in diameter, and projecting a few millimeters above the surface of the surrounding mucosa. These nodules were sharply circumscribed, were apparently situated in the submucosa and

* From the Division of Laboratories, Western Pennsylvania Hospital, Pittsburgh, and the Division of Laboratories, New York Hospital.

had a homogeneous white cut surface. At the upper border of the head of the pancreas, an ovoid, whitish nodule was found, measuring 5 by 3 by 3 mm. and projecting above the adjoining surface of the organ from 2 to 3 mm. The cut surface of this nodule was white and homogeneous.

*Pancreas.*—Sections were taken from various portions of the pancreas.

In an indistinctly circumscribed area, the alveolar structure of the organ could not definitely be recognized. At the periphery of this area, the glandular epithelium still had an alveolar arrangement. But toward the interior of this area the alveolar arrangement was seen to be gradually lost. Like the islands of Langerhans, the

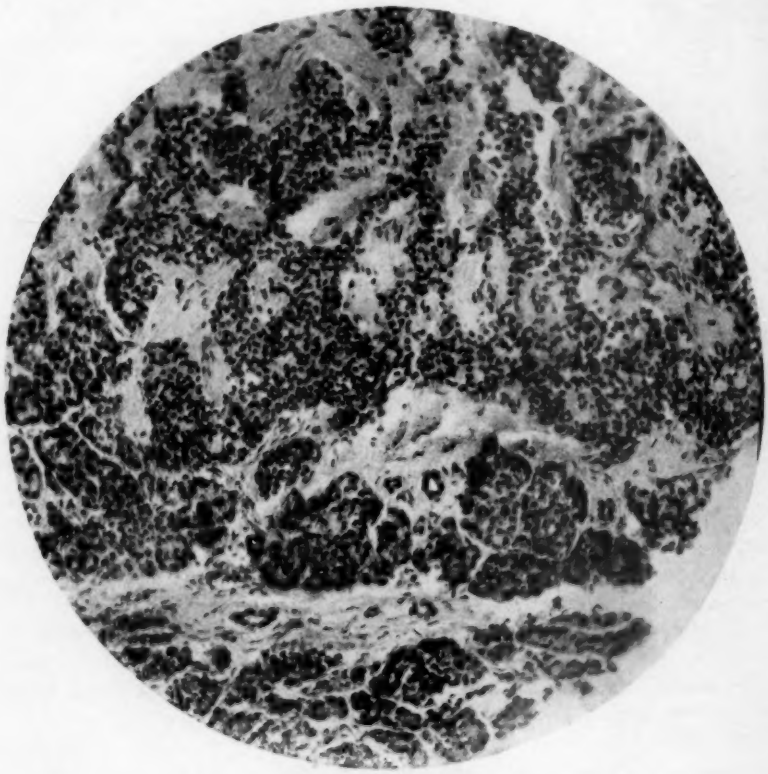

Fig. 1.—Hamartoma of the pancreas.

greatest part of this region was made up of cords and solid nests of polygonal cells with a pink-staining cytoplasm.

The yellowish nodule on top of the head of the pancreas, mentioned in the gross report, was separated from the pancreas by a loose connective and fat tissue, which did not differ from the connective tissue septum between the lobules of the pancreas (fig. 1). The periphery of this nodule was made up of typical alveolar pancreatic tissue. Thus, what was considered grossly to be a nodule on the top of the pancreas, on account of its peripheral pancreatic structure, appeared to be an accessory pancreatic lobule. A fact likewise favoring the latter interpretation was the presence of glandular ducts, lined by one layer of columnar cells, in the supporting stroma of this nodule. The chief part of the nodule was made up of

nests and anastomosing cords of epithelial cells. These cells, about the size of the epithelial cells of the islands of Langerhans, were polygonal, with a faintly pink, spongy cytoplasm. At the periphery of the epithelial cords, some cells were columnar, their nuclei were larger and oval and located in the middle of each cell. Some round spaces were seen within the epithelial cords, lined by somewhat flattened epithelial cells and filled with a homogeneous material staining faintly pink. In some portions, a similar amorphous substance surrounded the capillaries of the framework of connective tissue supporting the epithelial cords. In some other portions, the framework consisted of ordinary fibrous connective tissue. The

Fig. 2.—"Carcinoid" of the duodenum

amount of the latter, separating the epithelial cells from the capillaries, was scanty in many places. Consequently, in these instances, the epithelial cells lay in almost direct contact with the capillaries, as in the islands of Langerhans. In view of the fact that recently the presence of silver-reducing granules in the epithelial cells of the "carcinoids" of the alimentary tract had been repeatedly reported, an attempt to demonstrate such granules was made in the case under consideration. Sections were treated according to the method of Hasejawa. Silver-reducing granules were not demonstrated.

*Duodenum.*—Sections of one of the nodules in the duodenum showed that it was confined to the submucosa (fig. 2). It was separated from Brunner's glands

by a narrow strand of connective tissue. The muscularis mucosae was continuous with the network of connective tissue forming the stroma of the nodule. The architectural arrangement of the elements of this nodule was essentially the same as that described for the nodule on the head of the pancreas. The amount of stroma, however, was much smaller in the duodenal than in the pancreatic nodule. A peripheral zone of alveolar pancreatic tissue was not present in the duodenal nodule, but the number of alveolar structures within the epithelial nests and cords was larger in this nodule. They consisted here of a ring of cuboidal cells surrounding a minute, round lumen. The nuclei of these cells were located at the

Fig. 3.—Adenomyomas and adenomas in the muscularis of the duodenum.

cell base. The stroma did not contain any glandular ducts such as were found in the nodule on top of the pancreas. The duodenal nodule was distinctly separated from the mucosa by strands of the muscularis mucosae.

In addition to the gross nodule described, on histologic examination, several nodular structures were found in the muscular layer of the descending part of the duodenum (fig. 3). Each of these consisted essentially of a group of glandular ducts. Most of the ducts were lined by one layer of undifferentiated columnar cells. In some instances, a duct was lined by goblet cells, similar to those of the intestinal epithelium. Each nodule was surrounded by concentric layers of connective tissue, branches of which, entering the interior of the nest, formed a supporting network. The adjoining muscular tissue was free of evidence of any

inflammatory reaction. Some of these nodules were located in the immediate vicinity of Brunner's glands. They could be easily differentiated from Brunner's glands, however, by the staining reaction of the cytoplasm of their cells. Evidence of a transition from Brunner's glands into these glandular groups was not found.

COMMENT

*Duodenum.*—A well circumscribed nodule of epithelial tissue was found in the submucosa of the duodenum. In view of this heterotopic epithelium, the question arises: Was it a malignant epithelial neoplasm? A carcinomatous nature for similar structures has repeatedly been claimed, but the evidence has never been generally accepted, which accounts for the use of the term "carcinoid." Burckhardt [1] described and discussed structures closely resembling these. He found them in the jejunum and the ileum and was able to demonstrate repeatedly a direct union between solid strands of tumor and the epithelium of Lieberkühn's crypts, a union which he assumed to be a true transition. This assumption, he believed, was supported by the fact that the crypts of Lieberkühn of these regions were lined by definitely changed, tall, hyperchromatic cells. He further noted that in all of his cases the muscularis mucosae was infiltrated by the tumor, and therefore he regarded the latter as a relatively benign carcinoma. Krompecher [2] regarded the majority of the "carcinoids" of the small intestine and the appendix as identical with his "basaliomas" because both of them form nests and cords consisting of small hyperchromatic cells with a small amount of cytoplasm and because they infiltrate the tissues. To support this interpretation of the majority of the carcinoids, Krompecher brought forward the following considerations:

He claimed that the multiplication of the cells of the intestinal epithelium takes place in the lower portion of Lieberkühn's crypts; accordingly, he regarded the cells of that region as basal cells corresponding with the layer of basal cells in a squamous cell epithelium. These "basal cells" of Lieberkühn's crypts are, in his opinion, the matrix of most of the carcinoids of the small intestine. Krompecher himself, however, reported a case which, in his opinion, could not be interpreted in this way. He found a tumor of the small intestines, the size of a hazelnut, which histologically consisted of an incompletely differentiated anlage of the pancreas, resembling a basalioma. In view of the regular configuration of the cells, the presence of lumina surrounded by well oriented columnar epithelium, and the absence of an infiltrative growth, Krompecher, in this instance, excluded the diagnosis of basalioma.

The same arguments excluded a diagnosis of basalioma or of a "relatively benign carcinoma" in the case under consideration.

1. Burckhardt, J. L.: Frankfurt. Ztschr. f. Path. **3**:593, 1909; **11**:219, 1912.
2. Krompecher: Beitr. z. path. Anat. u. z. allg. Pathol. **72**:163, 1924.

Here a transition of "tumor" strands into Lieberkühn's crypts was not observed. The mucosa covering the tumor did not show any significant change. Since the structure was not a glandular carcinoma of the intestinal mucosa and was not a so-called basalioma, it cannot be regarded as a neoplasm taking its origin from epithelial elements of the adult intestinal mucosa.

The structure of the duodenal nodule under consideration resembled that of the islands of Langerhans. In view of this similarity, which is often mentioned in the literature covering the subject, the question arises whether any relationship exists between such carcinoids and the islands of Langerhans or the anlage of these islands. The anlage of the pancreas is formed by a certain portion of the entoderm of the first or anterior part of the fetal gastro-intestinal tract. If the processes of growth and differentiation of the gastro-intestinal tract do not occur in normal chronologic sequence, a group of cells destined to become pancreatic tissue may be carried away from its normal location and become aberrant. Since the anterior part of the fetal gastro-intestinal tract is fixed at the cardia and at the ductus vitellinus, such misplacements of anlagen can naturally not occur beyond these points of fixation. The present nodule, being located in the duodenum, may be interpreted as being due to such a misplacement of an anlage.

In a large percentage of the cases reported, however, such "carcinoid" nodules have been found in the lower part of the ileum. Saltykow,[3] in 1912, described such a structure in the sigmoid flexure and another one in the submucosa of the rectum. In such cases, the assumption of a misplacement of an anlage appears hardly justifiable. The occurrence of carcinoids, such as are present in this case, throughout the entire gastro-intestinal tract rather leads to the assumption that the fetal entoderm of the entire gastro-intestinal tract is able at certain times to produce pancreatic tissue for unknown reasons.

Lauche,[4] in his work on epithelial heterotopy in the alimentary tract, presupposed such an ability on the part of the entoderm. He showed that, with this hypothesis, a series of tumor-like structures of the gastro-intestinal tract is explainable as the result of a dysontogenetic heterotopy. He emphasized the fact that epithelial buds of the entoderm have frequently been found in human, as well as in animal fetuses. In the majority of instances, such epithelial buds probably assume normal relations to the intestinal epithelium during the course of the ontogenesis. If this does not occur, these epithelial buds differentiate more or less completely, in various directions. Lauche expressed the opinion that the nature of the surrounding tissue exerts a deciding influence as to

3. Saltykow: Beitr. z. path. Anat. u. z. allg. Pathol. **54**:559, 1912.
4. Lauche: Virchows Arch. f. path. Anat. **252**:39, 1924.

the direction of differentiation. If a secretory duct connected with the intestinal lumen is present, according to Lauche, the so-called "organoids," like the adenomyoma, the adenoma of the intestinal gland type and the accessory pancreas, are formed. If such a secretory duct is not present, cystic structures are formed, proliferations of ducts and islands alone. Finally, Lauche considers epithelial buds which remain solid to be the point of origin of basal-cell proliferations. According to his opinion, the carcinoids are partly this type of "basal-cell proliferations" and partly proliferations of islands (Langerhans). He furthermore claimed that the majority of the carcinoids of the appendix are not proliferations of islands, whereas the latter do certainly occur in the small intestines. A genetic relationship between structures such as the accessory pancreas and the adenoma of the intestinal gland type is, according to Lauche, demonstrated by the fact that in an adenoma of this type which he found, he observed, in the deeper parts, channels which assumed the appearance of ducts. They were surrounded by incompletely differentiated pancreatic lobules.

While in the case under consideration the location of the submucous nodule of the duodenum allows one to interpret it as being due to a misplacement of an anlage, it remains to be seen whether a similar explanation may apply to the rest of the observations.

The muscularis of the duodenum contained, close to each other, groups of heterotopic epithelium, which were differentiated in various directions; partly as "adenomyomata," partly as "adenomata of the intestinal gland type."

The examination of these various heterotopic epithelial structures in the duodenum did not lead one to a tenable histogenetic interpretation. The observations in the pancreas, however, were of some significance in this connection.

*Pancreas.*—The structure of the peripheral zone of the nodule on the head of the pancreas suggested a pancreatic lobule. In interpreting the island-like proliferation of the central part of this lobule, it appeared necessary to take into consideration the topography of the normal island of Langerhans and its behavior toward surrounding structures.

Nakamura,[5] in his "observations on the fetal pancreas," emphasized the fact that all observers regularly have encountered an island in the center of the primary pancreatic lobule. He himself reported two cases, one that of a 6 months old infant and the other that of a 3 years old child, in which the central part of some pancreatic lobules consisted entirely of island tissue and was surrounded by only a narrow zone of tubular tissue. His description is almost literally applicable to the present case. In view of this complete topographic and considerable

5. Nakamura: Virchows Arch. f. path. Anat. **253**:290, 1924.

structural resemblance, a genetic relationship between the island-like proliferation in the present case and a primary island of Langerhans appears probable.

Is it a neoplastic proliferation of cells originating from the central island of a primary pancreatic lobule? If this is the case, the neoplasm growing in an expansive manner and gradually rising above the surface of the organ, would necessarily exert pressure on the peripheral glandular zone of the lobule. The microscopic examination of the peripheral tubular zone, which distinctly projected above the surface of the organ, failed, however, to reveal evidences of such pressure. On the contrary, the peripheral glandular zone morphologically had to be regarded as a part of the nodule under consideration. These observations fairly exclude the conclusion that a true neoplastic process is represented in the nature of the proliferation of the central island.

One might assume that the peripheral glandular zone of the nodule had secondarily grown over the surface of the nodule. Since, however, "islands," as well as peripheral glands, morphologically are parts of one topographic unit, and since a connection of the glandular zone with the parenchyma of the rest of the pancreas was not found, the nodule has to be regarded as corresponding with one pancreatic lobule, an interpretation which excludes the aforementioned assumption. These considerations lead to a third interpretation: The peripheral glandular zone took its origin from the central "island proliferation." In other words, the present case can be interpreted only on the assumption of an insulo-acinar transformation.

According to Seyfarth,[6] the frequent finding of a central island in the lobules of the fetal pancreas points to an insulo-acinar transformation. Weichselbaum and Kyle [7] occasionally found in the periphery of larger islands of the fetal pancreas a lumen lined by regular epithelium. In the present case, too, similar lumina were found in the peripheral parts of the "island" proliferation, and various stages of the formation of such lumina in the epithelial cell cords were observed. These observations also point toward an insulo-acinar formation. Further support for such an interpretation is found, according to Seyfarth, in the observations made in congenital syphilis. If, in such cases, "the lesion starts early enough, the islands are differentiated, but a further transformation into tubules does not take place" (Nakamura).

Returning now to the question of the nature of the nodule on the head of the pancreas, I hold that a proliferating central island of a primary pancreatic lobule has to be regarded as the point of origin of the structure under consideration. This proliferation of the island

6. Seyfarth: Neue Beitrage zur Kenntnis der Langerhans-Inseln, Jena, 1920.
7. Weichselbaum and Kyle, quoted by Nakamura (footnote 5).

showed a subnormal tendency to differentiate as glandular tissue. This behavior may be interpreted as an incomplete differentiation of this part of the pancreatic anlage. In other words, one is dealing with a hyperplastic, neoplasm-like malformation of tissue, a "hamartoma" (E. Albrecht).

As has been described, the pancreas showed, apart from this hamartoma, evidence of a disturbance of the ontogenesis. In one of the pancreatic lobules, the proportion of parenchyma to stroma was the same as in the fetal pancreas. In another part of the pancreas, the alveolar structure was not fully developed.

## COMPARISON OF THE GROWTHS IN THE DUODENUM AND THE PANCREAS

In order to arrive at an interpretation that will cover both growths, that in the duodenum and that in the pancreas, it is necessary to compare the observations made in the pancreas with those made in the doudenum. The submucous nodule of the duodenum, apart from the absence of a peripheral glandular zone, corresponded with the hamartoma of the pancreas in its main features. The hamartoma of the pancreas corresponding topographically with a lobule of the pancreas cannot be regarded as being due to a misplacement of an anlage. Accordingly, an interpretation common to the duodenal as well as to the pancreatic nodule is that they are neoplasm-like malformations of tissue, hamartomas, arising out of faulty differentiation of the entoderm, in the sense of Lauche. Faulty differentiation of the entoderm may also account for the group of ducts and intestinal glands in the duodenal muscularis.

A faulty differentiation of the entoderm led to various other malformations: heterotopic in the wall of the duodenum, homoiotopic in the pancreas. The remarkable resemblance between the hamartoma of the pancreas and the submucous nodule of the duodenum may appear less inexplicable if it is considered that in both cases one is probably dealing with a faulty differentiation of a matrix common to both.

## SUMMARY

In the present case, a tumor nodule was found on the head of the pancreas. On the basis of its topography, its structure and the behavior of the adjoining pancreas, this nodule is interpreted as a neoplasm-like malformation of tissue, due to an incomplete insulo-acinar transformation of a primary pancreatic nodule.

In the same case, heterotopic epithelial structures in the wall of the duodenum were found. The muscular coat contained adenomyomas and adenomas of the intestinal gland type. The submucous coat con-

tained a carcinoid. In spite of the heterotopy, none of these epithelial structures showed the morphology of a malignant epithelial neoplasm.

The malformation of the pancreas, the adenomyomas and the adenomas of the intestinal gland type in the muscularis of the duodenum appear to have been due to a faulty differentiation of the entoderm.

Evidence of dysontogenesis and absence of signs of malignancy led the observer to regard the carcinoid of the duodenum as a dysontogenetic heterotopy.

# THE MICROSCOPIC CHANGES OF HETEROPHILE SKIN REACTION

## THE PATHOLOGY OF HETEROPHILE SKIN REACTIONS *

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In a paper published in 1926,[1] a typical and rapidly appearing reaction of the skin was described, which occurs when rabbit serum containing a demonstrable content of heterophile antibody is injected into the skin of a normal guinea-pig. As reported in that article, noticeable changes take place within a few minutes after the injection and proceed rapidly to a characteristic and well defined lesion. Control injections of rabbit serum in which a heterophile antibody has not been generated, or of serum from which the antibody has been adsorbed by its specific antigen, do not give such reactions.

From the results described, it appeared that the changes noted in treated areas of the skin depend on the reaction of the antibody and its specific antigen within the tissues. For this reason, it seemed advisable to study the reaction by histologic methods, in order to learn, if possible, something of the minute changes taking place and their significance. Advantage was taken of the early appearance and progressive character of the reaction. A careful comparative study has been made by the microscopic examination of typical lesions excised and fixed at progressive intervals following injections of the antiserum.

Throughout the literature the study of reactions of the skin seems largely to have been confined to gross observations and to macroscopic descriptions involving the appearance or nonappearance of local changes following treatment with various protein and nonprotein substances. Minute microscopic studies of reactions of the skin when antigen and antibody are involved have been reported in few cases. Such a study of the Arthus phenomenon was made by Opie [2] in 1924. Strickler,[3] in 1922, reported a small series of cases in human beings in which areas that gave positive reactions to the tuberculin and luetin tests were

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* From the Otto Baer Fund for Clinical Research and the Nelson Morris Institute for Medical Research of the Michael Reese Hospital, and the Department of Medicine and the Douglas Smith Foundation for Medical Research of the University of Chicago.

1. Redfern, W. W.: A Study of the Primary Toxicity of Heterophile Immune Rabbit Serum for Guinea-Pigs, and Its Apparent Relation to the Phenomenon of Anaphylaxis, Am. J. Hyg. **6**:276, 1926.

2. Opie, E. L.: Inflammatory Reaction of the Immune Animal to Antigen (Arthus Phenomenon) and Its Relation to Antibodies, J. Immunol. **9**:231, 1924.

3. Strickler, A.: A Histopathologic Study of Positive Cutaneous Tests, J. A. M. A. **78**:1287 (April 29) 1922.

excised from patients and studied microscopically. A series of local reactions occurring after the intradermal injection of certain food extracts was also included. A second series of similar cases was reported by Strickler and Asnis [4] in 1923. Excisions of the tissues in these cases were made forty-eight hours after the injections of the materials, at a time when the reactions were considered to be at their height. These authors report certain differences which they consider constant in the character of the cellular response in reactions to tuberculin and luetin. The results following injections of food extracts, however, were irregular and did not show typical characteristics.

In the use of heterophile antibody-containing serum injected into guinea-pigs, I feel that the problem is being approached from a somewhat more basic standpoint. The antigen is known to be present in the cells of the guinea-pig. The antibody can be demonstrated in the serum used, and its concentration can be regulated. Hence, in this reaction the results can be safely interpreted as due to the interaction of these specific bodies.

## EXPERIMENTAL PROCEDURE

A rabbit serum having a high hemolytic titer for sheep corpuscles was produced by the injection of boiled sheep corpuscles. The antibody was, therefore, heterophile (Forsman [5]). The titer of this serum was about 20,000.[6] This serum when injected intradermally into the shaved abdomen of a normal guinea-pig gave rise to the typical reaction, as previously described. Quantities of 0.2 cc. were injected in all instances. A control injection of normal rabbit serum accompanied each injection of heterophile antiserum at a different site on the abdomen of the same animal. Excision and fixation of both areas, including the skin and underlying muscle, was performed under anesthesia at the same time, and paraffin sections were made for microscopic comparison. The serums used were uncontaminated and did not contain chemical preservatives.

Excisions were made at intervals of 10, 25 and 45 minutes, and 1, 2, 6 and 24 hours. The animals used were white normal guinea-pigs weighing about 250 Gm. One injection and one control injection were made in each animal. Confirmation of the results of this series was obtained by the injection of a second and third similar series, time intervals of 30 minutes, 1 hour, 2 hours and 6 hours elapsing between injection and excision.

The changes observed in the test areas of each period were compared with those of the control areas and were, briefly, as follows:

Period 1 (ten minutes after injection).—At this time the differences were not marked. In both test and control injections, the tissue structure had been ruptured by the introduction of serum into the corium. In so short a time as ten

4. Strickler, A., and Asnis, E. J.: The Histopathology of Cutaneous Tests, Arch. Dermat. & Syph. **7**:379 (March) 1923.

5. Forsman, J., and Fex, J.: Ueber heterologe Antisera, Biochem. Ztschr. **61**:6, 1914.

6. That is, 0.1 cc. of a 1:2,000 dilution hemolyzed 0.1 cc. of a 10 per cent suspension of washed sheep corpuscles in the presence of 0.1 cc. of a 20 per cent dilution of fresh guinea-pig serum.

minutes, little absorption of the serum had occurred; hence, the amount of edema apparent in the sections was about the same in each case. This was referred to as passive edema, in contradistinction to the active edema observed after the lapse of a longer period. The average number of capillaries in the corium which were distended with blood was about the same in both the test and the control areas. An active state of vasodilatation could not be determined in either case.

At this early stage, however, two points of difference were distinguished.

The first of these was the early indication of petechial hemorrhage. In the test sections there were a few small vessels, outside which small aggregates of erythrocytes appeared. This condition did not occur in the control series. Accompanying the hemorrhage, a few small thrombi were seen, forming already along the walls within the vessel. Not every section showed these changes, and only a few were observed in all the ten minute sections.

The second point of difference was a changed appearance in the connective tissue fibers of the corium. These seemed to have lost their sharp definition, and many presented the appearance of homogeneous, eosin-staining masses. This impression was strengthened by the examination of tissues subjected to the antiserum for longer periods. This is believed to be an evidence of cytoplasmic injury and to represent the earliest change which goes on to necrosis.

PERIOD 2 (from twenty-five to thirty minutes after injection).—In this set the changes observed were not much different from those in the ten minute period. The constant observations were: slight petechial hemorrhage, capillary thrombi more numerous than in the previous set and the appearance of degenerative change exhibited by the connective tissue elements of the corium.

PERIOD 3 (forty-five minutes after injection).—By this time, the tissue which received the antibody showed much hemorrhage, with diffusion of erythrocytes throughout the corium in the treated area. Many thrombi were present in the capillaries and small vessels. In certain places, sections of these vessels were observed, the walls of which were definitely ruptured, with erythrocytes flowing through into the surrounding spaces. In the controls, however, perivascular infiltration of leukocytes was pronounced, but a definite rupture or outflow of red cells was not seen. At this stage, also, the corium of the treated area appeared decidedly necrotic, the collagenous elements having a hyaline appearance and the nuclei being pyknotic. The amount of edema was not yet markedly increased.

PERIOD 4 (two hours after injection).—This stage was marked by heavy infiltration of the entire area with red corpuscles, and the majority of the capillaries contained thrombi which filled the lumina. These had the appearance of fibrin and not of hyaline thrombi. The necrotic appearance noted before was still evident. The controls, as those of the previous sets, did not show thrombosis of capillaries or ecchymosis, although infiltration with white cells was much more pronounced than it was in the test areas. At this stage, also, active edema began to be manifested. The spaces filled with fluid were slightly more enlarged in the test area than in the control area. This difference was confirmed also by macroscopic examination of the areas prior to fixation, and could be detected in the gross somewhat earlier than with the microscope. Figures 1 and 2 illustrate some of these differences.

PERIOD 5 (six hours after injection).—These tissues differed from the two hour series only by an increase in the amount of edematous fluid localized about the treated area. The control did not show such an increase, but rather a diminution, owing to the absorption of the injected serum.

PERIOD 6 (twenty-four hours after injection).—Twenty-four hours after injection in both the test and the control areas, subcutaneous edema was pro-

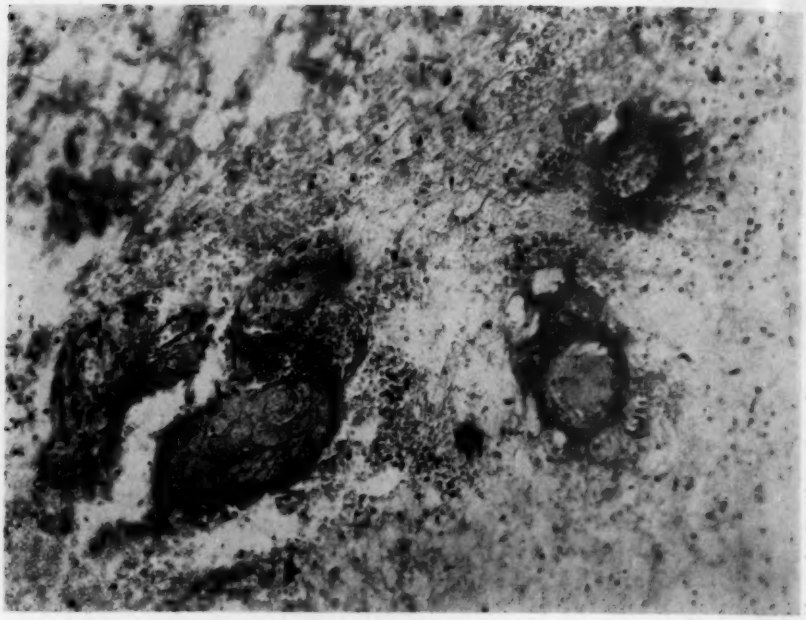

Fig. 1.—A typical low power field in the test area two hours after injection of the antiserum. The vessels are occluded by fibrin thrombi, while surrounding tissues contain many erythrocytes. The absence of leukocytes from this area should be noted.

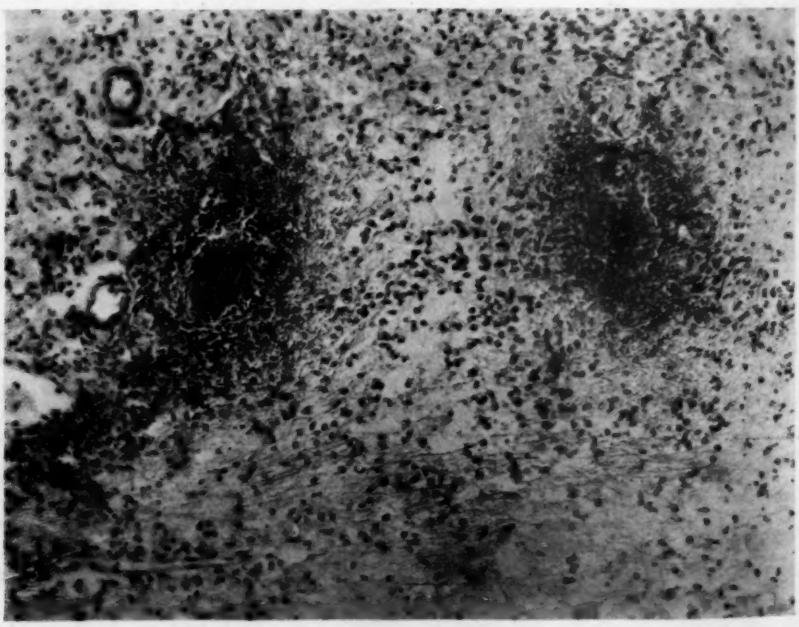

Fig. 2.—A typical field from the control area two hours after injection. The vessels contain erythrocytes, while none are seen outside. A heavy infiltration of leukocytes surrounds the vessels.

nounced, and inflammatory repair processes were seen. Young proliferating fibroblasts were scattered through the edematous area. The significant difference at this time was in the distribution of the elements of the blood. Test specimens still showed many scattered red cells throughout the region and a few thrombi, whereas these were not seen in the control areas. Both were thickly studded with white cells, but there was a distinct difference in the types observed. The control area showed by far the larger number of polymorphonuclear leukocytes, while in the test area large mononuclear types predominated. The exact significance of this is unknown, but it has been observed rather constantly.

## COMMENT

In interpreting the early changes as observed, it is of interest to determine which structures are affected by the presence of the specific antibody. That is, what are the immediate results of antigen-antibody union within the tissues? In this one supposes that it is such a union and not simply the presence of a toxin in the antiserum which causes the injury to cells. This supposition can be based only on the fact that such antiserum does not produce changes in rabbits and other animals of the rabbit type.

The most definite and constant result appears to be injury to the vessel walls. Loss in continuity of the endothelial lining of a vessel is difficult to demonstrate microscopically. However, the observation of many points where minute vessel walls are apparently ruptured, and where erythrocytes can actually be seen leaving the vessel, forces the conclusion that there is a true break in continuity rather than simply a loss in function on the part of the endothelial cells. Control sections showed many leukocytes both inside and outside the vessels, and even in the process of passage through the lining, while the red cells were always within the lumen. This fact also eliminates the technic from responsibility for the injury.

The other constant observation in connection with the capillaries was the formation of fibrin thrombi in the test areas. These corresponded in number and development in direct ratio with the increase in the amount of ecchymosis seen in the test sections. The control sections did not exhibit in any instance either hemorrhage or thrombosis. As was noted before, the early sections showed merely a few small thrombi in process of formation attached to the walls and occupying only a fraction of the vessel lumen, whereas at later periods most vessels in the field contained fibrin plugs which filled or nearly filled the lumina.

In his study of the Arthus phenomenon previously referred to, Opie [2] reported the observation of hyaline thrombi which he considered to be composed of degenerating erythrocytes. In my material, however, the thrombi were distinctly fibrinous. Erythrocytes were enmeshed in some of these but without showing signs of degeneration. Moreover, my observations were made on changes which take place within a few

minutes, whereas the changes seen in the Arthus phenomenon are apparent only after the lapse of several hours.

As a result of these constant observations two possibilities present themselves as explanations for the capillary injury and consequent extravasation of erythrocytes:

1. The specific combination of antigen and antibody injures the cells of the vessel walls, allowing ecchymosis, with the incidental subsequent formation of fibrin thrombi at sites of injury.

2. The specific antibody brings about changes in the serum components which precipitate fibrin formation, and the ecchymosis results only from plugging of the vessels. Thus, the necrotic changes in the area might be explained as a result of dysfunction of the blood supply through occlusion of the vessels by fibrin thrombi. If this were true, the injury observed would not be a direct but a secondary effect of the antigen-antibody combination.

The possibility that the antiserum might be active in precipitating fibrin in the plasma was tested by some simple experiments in vitro. Two serologic tubes were coated inside with paraffin. In one was placed 0.5 cc. of serum from a normal rabbit and in the other 0.5 cc. of rabbit serum having a high heterophile titer. A normal guinea-pig was bled from the heart, and 2 cc. of the freshly drawn blood was allowed to flow into each tube. This was mixed with the serum contained therein, and the clotting time for each tube was carefully recorded. This procedure was repeated with the blood of four other animals. The results are shown in the table.

*Effect of Heterophile Antiserum on the Clotting Time of Guinea-Pig Blood*

| Guinea-Pig | Clotting Time of Blood in | |
|---|---|---|
| | Heterophile Antiserum | Normal Rabbit Serum |
| A | 3 minutes 10 seconds | 4 minutes 15 seconds |
| B | 4 minutes 50 seconds | 5 minutes 10 seconds |
| C | 6 minutes 15 seconds | 6 minutes 10 seconds |
| D | 2 minutes 50 seconds | 2 minutes 20 seconds |
| E | 3 minutes 40 seconds | 5 minutes 5 seconds |

The series in the table indicates that the antibody in question does not have any effect on coagulation. The two instances in which differences of more than one minute were recorded are not regarded as significant, since such a difference was not observed in the remaining determinations.

The possible rôle of thrombosis assumed greater importance because of results reported by Kyes and Strauser.[7] They presented evidence

7. Kyes, P., and Strauser, E. R.: Heparin Inhibition of Anaphylactic Shock, J. Immunol. **12**:419, 1926.

from which they concluded that the symptom complex involved in anaphylactic shock in birds was due directly to the effect of multiple capillary thrombosis, which resulted from the protein injected as anaphylactogen. They showed that when the blood of birds was rendered incoagulable by the intravenous injection of heparin the anaphylactic syndrome was completely prevented. Hyde [8] attempted to apply this observation to the study of anaphylaxis and primary serum shock in guinea-pigs. He found that in this species the use of an anticoagulant substance was without effect in preventing fatal shock. He found also that the toxic effects of heterophile antiserum for guinea-pigs were not decreased by the use of heparin.

Having observed capillary thrombosis so constantly in the reactions in the skin I determined to test the importance of this factor in the reactions. Accordingly, injections were made in the skin in a new series of animals which had previously received heparin intravenously, and the reactions were studied at progressive periods as before.

In these cases, 0.5 cc. of a 1 per cent solution of heparin per hundred grams of guinea-pig was used. This amount is greatly in excess of the quantity reported to be necessary to prevent coagulation of the blood in vivo. The material was injected into the hearts or jugular veins of the animals, and assurance that its anticoagulant effects were being secured was gained by the withdrawal of a few drops of blood from the ear of the animal every half hour thereafter. By this means I found, as did Hyde, that coagulation was completely prevented for about four or five hours. Fifteen minutes after the injection of heparin, injections were made in the skin exactly as previously described, a test and a control injection being made in each animal. Macroscopic observations and the microscopic study of sections from these areas yielded some interesting results.

Macroscopically, the skin lesions in these animals seemed to develop a bit more slowly than they did in the animals not treated with heparin. The approximate degree of change seen in the normal animal in one hour was not reached in the heparinized pig until about one and a quarter hours after injection. However, the reactions appeared typical in every respect and progressed as did the others.

Microscopically, thirty minutes after the injection of the serum into a heparinized animal, some of the vessel walls were seen to be ruptured, with a few red cells free in the surrounding tissues. This picture was about the same as that shown by the nonheparinized tissues removed at the same time.

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8. Hyde, R. R.: Heparin Inhibition of Anaphylactic Shock, Am. J. Hyg. 7:614, 1927.

After one hour, slightly more hemorrhage was seen, and the necrotic appearance of the connective tissue fibers was becoming evident.

After two hours, the reaction was most pronounced, and the interstices were filled with erythrocytes. In all these reactions, however, thrombi were not found. Evidence of injured vessel walls was indicated by poor staining of endothelial nuclei and by many ruptured areas showing extravasation in progress (fig. 3).

The six hour series showed a diffuse infiltration of red cells throughout the area. Most of the vessels were still free from thrombi, but in a few well formed fibrin clots were present. By this time, as indicated before, the peripheral blood when drawn clotted, showing the effect of heparin to have largely disappeared.

Fig. 3.—A typical microscopic field from a heparinized animal two hours after the intradermal injection of heterophile antiserum. The great extravasation of erythrocytes without the presence of thrombi should be noted.

From these results, it seems certain that capillary thrombosis is not a primary factor but actually a secondary result of extensive injury to the vascular endothelium. This would tend to confirm the results of Hyde in the matter of fatal shock, and to show that the antibody injected brings about an actual injury to tissue elements with which it comes in contact. The fact that vessel linings suffer most acutely is made evident both in the local reactions and in the general shock when pulmonary edema has been shown to be most constant. The expla-

nation is supported by the results of Halber [9] who demonstrated that in both the horse and the guinea-pig the cells which line the vessel walls are rich in heterophile antigen. This was shown by means of the cellular material carefully scraped from the inner surface of the aorta.

## CONCLUSIONS

The facts of general interest which have been learned from these investigations may be briefly summarized. In the reactions of the skin the evidence is clear that injury and death of the cell take place rapidly after the antigenic and antibody factors have combined. That neither factor is, of itself, toxic has been shown. That a new substance which is toxic results from the reaction between antigen and its specific antibody seems possible, but this has not been clearly proved. This is entirely analogous to the matter of anaphylatoxin formation in the plasma, as postulated by Friedberger [10] and others. The formation of such a new separable substance has, however, never been satisfactorily established. Thus far, in some few attempts along this line I have been unable to produce a toxic substance by the combination of heterophile antigen and antibody in vitro.

In previous work of this sort there has been much discussion as to the site at which the union of antigen and antibody takes place. Many theories involving the ideas of sessile and free receptors have been evolved. In this instance we can demonstrate the antigenic unit as a component of the cell itself and the antibody as a single active entity. It is most simple and reasonable then to believe that the reaction between these two bodies takes place within the cell, especially since the early injury and death of the latter can be seen. This interpretation is indicated by the rapidly appearing injury to endothelial cells which then allow the escape of erythrocytes from the vessels. These same erythrocytes, which do not contain the antigen, do not show evidence of lysis or destruction and remain normal in appearance outside the vessels for many hours.

Another interesting observation which was but briefly mentioned was the apparent lack of chemotactic power exhibited by the immune serum, in contrast to the strong leukocytic response when normal serum was injected. A further investigation of this point is being made, as it has

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9. Halber, Wanda: Untersuchungen über das Forsmansche Antigen im Zusammenhang mit der Theorie der Entstehung der Antikörper, Ztschr. f. Immunitätsforsch. u. exper. Therap. **39**:282, 1924.

10. Friedberger, E.: Weitere Untersuchungen über Eiweissanaphylaxie, Ztschr. f. Immunitätsforsch. u. exper. Therap. **4**:636, 1910; Neuere Untersuchungen über den Mechanismus der anaphylaktischen Vergiftung mit besonderer Berücksichtingung der Anaphylatoxinvergiftung, Ztschr. f. Immunitätsforsch. u. exper. Therap. **18**:227, 1913.

been shown by Spät [11] that guinea-pig leukocytes are likewise carriers of the heterophile antigen.

Studies are still in progress, and the present communication is in the nature of a preliminary report. The work is being continued with attempts to study the mechanism of reaction within the living cell.

SUMMARY

A microscopic study was made of the reaction produced by the injection of heterophile antiserum into the skin of the normal guinea-pig. The early changes observed were capillary hemorrhage and extensive thrombosis of the minute vessels. Beginning necrotic degeneration of connective tissue elements was also evident.

When coagulation of the blood was prevented by the use of heparin, the reactions developed as usual and ecchymosis was still pronounced. This indicated acute early injury to capillary endothelial cells.

Control areas injected with normal rabbit serum showed early strong leukocytic response, while the sites in which antiserum was injected remained free from leukocytes for several hours.

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11. Spät, W.: Untersuchungen über ein Leukocytenimmunserum, Ztschr. f. Immunitätsforsch. u. exper. Therap. **21**:565, 1914.

# XANTHOMA OF THE CHOROID PLEXUS*

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As xanthoma of the human choroid plexus does not occur frequently, the following report of a case is deemed of interest.

A white man, aged 47, was admitted to the Pennsylvania Hospital (Dr. Newlin) with septicemia and arthritis of the right knee. He was comatose, so that his history was not obtainable.

The urine contained albumin. The leukocyte count was 8,150; erythrocytes, 4,100,000; hemoglobin content of the blood, per cent 75. The blood sugar content was 120, the blood urea 18.6 and the blood creatinin 1.4. The cerebrospinal fluid pressure was 6 mm. of mercury; the cell count was 17; the showing of globulin was negative; of sugar, positive. He died three days after admission.

The necropsy revealed staphylococcic arthritis of the right knee with septicemia, bronchopneumonia, infarcts and miliary abscesses in the brain. The liver showed biliary cirrhosis with fatty infiltration.

The choroid plexus of the lateral ventricles (fig. 1) was enlarged and studded with bright yellow masses, varying in size from that of a pinhead to that of a pea. Sections of the choroid plexus were stained with hematoxylin and eosin, cresyl violet, phosphotungstic acid hematoxylin, scarlet red, van Gieson's stain. Except for a single layer of choroid plexus cells which bordered the specimen and from which a few papillae projected, the normal structure had disappeared. The plexus consisted of a number of masses of closely packed cells separated by dense fibrous tissue containing dilated blood vessels and corpora amylacea. Scattered through the cell masses were many small triangular cavities which probably had contained free lipoid substances. The centers of the larger cell masses showed degenerative changes and cyst formation.

The cells in these tumors were large, flat and polyhedral, with small densely staining eccentric nuclei (fig. 2). The cytoplasm was finely granular, contained many vacuoles and, when stained with scarlet red, was found to be filled with lipoids.

The cells toward the center of the tumors were larger than those at the periphery and showed degenerative changes: dislocation and disappearance of the nucleus, and disappearance of parts of the cell wall. The vessels in the tumors were engorged, and in many places the perivascular spaces were infiltrated with small round cells.

The presence of large "foamy" cells in which the cytoplasm is filled with lipoid substances, and the deposits of free lipoids between the cells, indicate that this tumor is a xanthoma. Stewart [1] considers

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* From the Neuropathological Laboratory of the Pennsylvania Hospital, and the University of Pennsylvania School of Medicine, Philadelphia.

1. Stewart, M. J.: Xanthoma and Xanthosis, Brit. M. J. **2**: 893, (Nov. 15) 1924.

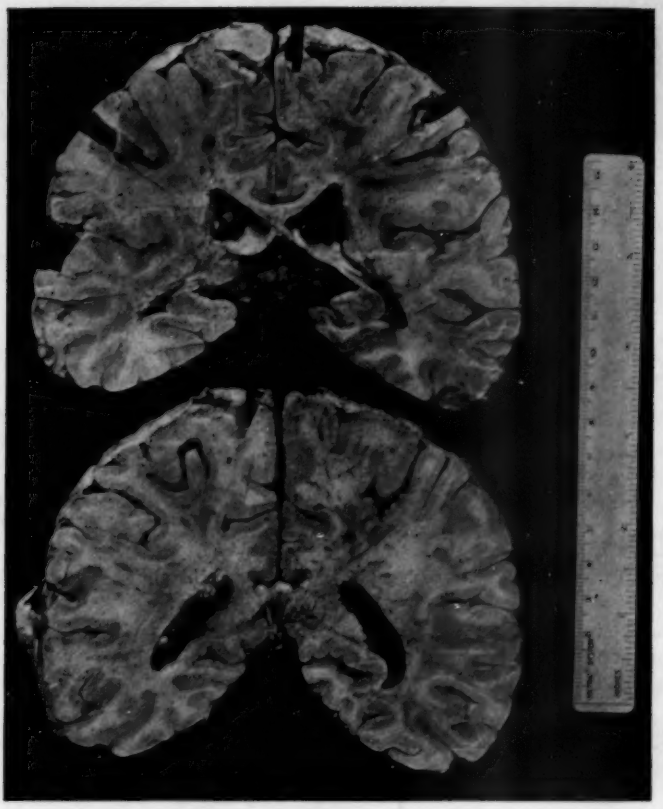

Fig. 1.—Xanthoma of the choroid plexus of the lateral ventricles. (In the lower section the choroid plexus of one side has been removed.)

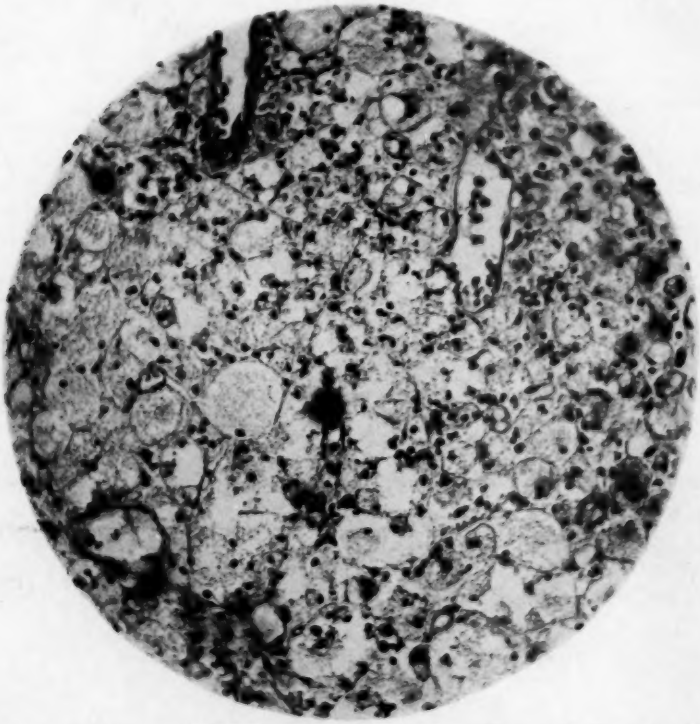

Fig. 2.—Section of a xanthoma of the choroid plexus, showing large polyhedral cells distended with lipoids. Hematoxylin and eosin; × 320.

that xanthomas of the choroid plexus, of the skin or of the gallbladder are the focal expression of hypercholesterolemia; and according to Beeson and Albrecht,[2] this hypercholesterolemia is due to retention of cholesterol as a result of hepatic dysfunction. The cholesterol is taken up by the endothelial cells of the choroid plexus, where it produces cell proliferation and eventually cell death. Some of the lipoid is deposited between the cells and excites an infiltration of round cells, among which giant cells may be present. Roussy [3] has given experimental proof of this concept by producing typical xanthomas by the injection of lipoidal material into the meninges of dogs.

Although an estimation of cholesterol was not made in this case, the presence of biliary cirrhosis with fatty infiltration of the liver indicates that hepatic function was disturbed and that hypercholesterolemia was the probable etiologic factor.

Xanthomas of the choroid plexus usually occur in the lateral ventricles, and seldom produce symptoms unless they attain sufficient size to cause obstructive hydrocephalus.

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2. Beeson, B. B., and Albrecht, P. G.: Contribution to the Study of Xanthoma Tuberosum, with Report of a Case, Arch. Dermat. & Syph. **8**: 695, (Nov.) 1923.

3. Roussy, G.: Les Cholesteatomes, Bull. de l'Assoc. franç. p. l'étude du cancer **5**: 192, 1912.

# THE STRUCTURE AND ARRANGEMENT OF THE BILE CANALICULI

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The application of the newer silver-impregnation methods of Cajal and Rio Hortega to the liver has enabled me to make a clearer demonstration of the intercellular bile canaliculi than has hitherto been possible and to add somewhat to the knowledge of their precise anatomic relationships. Existing literature on their minute anatomy and their relations to the hepatic cells, on the one hand, and to the terminal bile ducts in the portal spaces, on the other, is scanty and equivocal. Widely divergent statements are to be found in textbooks and published articles. The observations of histologists working with imperfect methods have been accepted or rejected according to individual opinion. The presence or absence of intracellular canaliculi, the nature of the canicular walls and the mode of union of these tiny passages with the excretory ducts are controversial questions. Concerning the last point, indirect evidence is strong that the bile ducts must be continuous with the intercellular network, but technical difficulties are so great that anatomists are still in doubt as to the exact character of this junction. Many textbooks maintain a discreet silence on the matter or dismiss it in a few words. Nevertheless, Beale [1] said that knowledge of the arrangement of the secretory structure of the liver must remain uncertain, unless this part of the course of the excretory ducts is conclusively shown.

## LITERATURE

Kölliker [2] (1854) was unable to demonstrate either the bile canaliculi or the mode of termination of the bile ducts. His opinion was essentially that of Jones [3] (1846), who believed the liver to consist of secretory parenchyma and excretory ducts discontinuous with each other and simply in juxtaposition. Bile was transmitted from cell to cell until it arrived in the area near the ducts, by which it was absorbed. Kölliker, however, stated that the inability of any one to show a direct communication between the hepatic cells and the bile

1. Beale, L. S.: Lectures on the Principles of Medicine, London, J. & A. Churchill, 1889.

2. Kölliker, A.: Manual of Human Histology, London, The New Sydenham Society, 1854, vol. 2, p. 111.

3. Jones, H., quoted by Kölliker (footnote 2).

ducts left an unfortunate hiatus in the knowledge of the minute anatomy of the parts which could hardly be made good by hypothesis. Gerlach [4] (1854), Budge [5] (1859), MacGillavry [6] (1864) and Chrzonszczewsky [7] (1866) showed, somewhat incompletely, that in various mammals (pig, sheep and rabbit) intralobular biliary passages existed which were capable of injection by various methods. The first description of these structures and their connections in man was given by Hering [8] (1872), who employed a mass injected into the common duct. It is by his name that the so-called "canals" at the points of transition have since been known. Kölliker and Rindfleisch [9] (1872) subsequently agreed with Hering. Eberth [10] (1867) found that with injections of gum and silver nitrate the walls of the canaliculi appeared doubly contoured. He therefore considered them to be lined with a separate membrana propria. By impregnation with a solution of alkanin in turpentine, Heidenhain [11] (1883) believed he could demonstrate a network of intracellular canaliculi in the livers of the rabbit and the frog. These he regarded as connecting the intercellular bile passages with small round intraprotoplasmic spaces previously injected by Pflüger [12] (1869) and von Kupffer [13] and since known as the secretion vacuoles of von Kupffer. While these results have been widely accepted, they have never been absolutely confirmed in the liver of man, although the same appearance has been many times produced by injection methods and has frequently been described in obstructive jaundice (Browicz,[14] Heyd, Killian and Klemperer.[15]

4. Gerlach, J.: Beiträge zur Structurlehre der Leber, Mainz, 1849.

5. Budge, J.: Ueber den Verlauf des Gallengange, Arch. f. Anat., Physiol. u. wissensch. Med. **26**:642, 1859.

6. MacGillavry, T. H.: Zur Anatomie der Leber, Wien. Sitzungsber. **1**:207, 1864.

7. Chrzonszczewsky, N.: Zur Anatomie und Physiologie der Leber, Arch. f. path. Anat. u. Physiol. **35**:153, 1866.

8. Hering, Edward, cited by Stricker: Human and Comparative Histology, London, The New Sydenham Society, 1872, vol. 2, p. 18.

9. Rindfleisch, G. E.: A Manual of Pathological Histology, London, The New Sydenham Society, 1872-1873, vol. 2, p. 82.

10. Eberth, C. J.: Untersuchungen über die Leber Wirbelthiere, Arch. f. mikr. Anat. **3**:423, 1867.

11. Heidenhain, R. P. H.: Handbuch der Physiologie, Leipzig, F. C. W. Vogel, 1883, vol. 5, p. 225.

12. Pflüger, E.: Ueber die Abhängigkeit der Leber von dem Nervensystem, Arch. f. d. ges. Physiol. **2**:459, 1869.

13. Von Kupffer, C., quoted by Heidenhain (footnote 11).

14. Browicz, quoted by Schaefer (footnote 23).

15. Heyd, C. G.; Killian, J. A., and Klemperer, P.: The Pathogenesis of Jaundice, Surg. Gynec. Obst. **44**:489, 1927.

Miura,[16] (1885) with the modified Golgi gold chloride method which he had previously described as demonstrating the reticular fibers in the liver, showed the delicate intercellular network without intracellular prolongations but he did not demonstrate the canals of Hering. Von Kupffer[17] (1889) also described a strain for the demonstration of the canaliculi. Beale[1] (1889), for whose opinion one must entertain the highest respect, entirely discredited the results of Hering and the German school, and, by injections of prussian blue, sought to show that the bile ducts were in direct continuity with what he called the "cell containing membrane" which entirely enveloped the hepatic cords. In his opinion, the fine intercellular tubules of Hering represented an incomplete filling of the biliary space within which the hepatic cells lay. Although subsequent work has shown Beale to be in error, he brought up one important point. He showed that the delicacy and extreme narrowness of the canals of Hering readily explain the difficulty in demonstrating the exact place where the bile ducts become continuous with the intralobular network. In the preparation of specimens, the tubes almost invariably break at these constricted points when attempts are made to isolate them.

Passing to more modern conceptions of these structures, one finds that Milne[18] described the canaliculus as being lined by an extremely fine flattened epithelium, which also forms the intermediate portion between the liver cord and the bile duct. According to Stöhr[19] and Krause,[20] the bile capillaries are minute tubes with continuous cuticular walls presumably formed by a local modification of the cell membranes of two adjacent hepatic cells. Stöhr regarded the so-called intracellular canaliculi as temporary phases of functional activity accompanying the discharge of secretion. The canals of Hering were not mentioned. Ellenberger[21] reproduced the injections of Frey, but added little concerning the cell relationships at the point of junction. Bohm and Davidoff[22] described this as a gradual transition from the cubical

16. Miura, M.: Beiträge zur Kenntniss der Gallencapillaren, Arch. f. path. Anat. u. Physiol. **99**:512, 1885.

17. Von Kupffer, C.: Zwei Methoden zur Tinction der Gallencapillaren und der intralobulären Fasern der Leber, München. med. Wchnschr. **36**:767, 1889.

18. Milne, L. S.: The Histology of Liver Tissue Regeneration, J. Path. & Bact. **13**:127, 1909.

19. Stöhr, Philip: A Textbook of Histology, ed. 2, Philadelphia, P. Blakiston's Son & Co., 1913, p. 285.

20. Krause, Rudolph: A Course in Normal Histology, New York, Ribman & Company, 1913, p. 213.

21. Ellenberger, W.: Handbuch der vergliechenden mikroskopischen Anatomie der Haustiere, Berlin, Paul Parey, 1911, vol. 3, p. 343.

22. Bohm, A. A., and Davidoff, M.: A Textbook of Histology, ed. 2, Philadelphia, W. B. Saunders Company, 1916.

epithelium of the bile ducts to the large hepatic cells. Schaefer [23] was inclined to agree with Heidenhain in regard to the presence of intracellular canaliculi but, other than the injection figure of Cadiat, he showed no histologic preparation of the Hering canal although he postulated its presence. Branca [24] described fully both the intracellular canaliculi and the Hering canals. The most recent study is that of Letulle [25] (1914). Using the iron-alum-hematoxylin method, he was able to give an excellent description of the intercellular canaliculi and to figure the canals of Hering clearly. At the junctional point, he found a transitional type of cell. Contrary to Heidenhain, Branca, and others, he stated flatly that intracellular canaliculi do not exist.

## METHOD

The method used in this study is an adaption of the Rio Hortega silver carbonate stain, for the details of which I am personally indebted to Dr. Perez Vincente Fontana. Small blocks of normal human liver are fixed for at least twenty days in 10 per cent solution of formaldehyde, and from these, frozen sections are cut as thin as possible. A number (five or six) are then alternately heated and cooled for twenty minutes in the silver carbonate pyridin bath [26] until they are uniformly golden brown. Care must be taken that heating does not reach boiling point; it should cease when steam arises from the surface. The sections are rapidly washed in distilled water and placed in 20 per cent neutral solution of formaldehyde for one minute, and finally fixed in a solution of 2 per cent sodium thiosulphate for from one-half to one minute. Thorough washing in tap water is then essential, better results being obtained if it is prolonged for two or three days. A little neutral solution of formaldehyde may be added with advantage. The sections are dehydrated in 95 per cent alcohol and absolute alcohol, cleared with carbol xylol, and mounted in Canada balsam.

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23. Schaefer, E. S., cited by Quain: Elements of Anatomy, London, Longmans, Green & Company, 1912, vol. 2, p. 563.

24. Branca, Albert: Précie d'histologie, ed. 5, Paris, J. B. Baillière et fils, 1921, p. 394.

25. Letulle, Maurice: Les capillicules biliares du foie humaine, leur repartition à l'antérieur du lobule hépatique, leurs modes d'abouchement dans les canaux perilobulaires, J. de physiol. et de path. gén. **16**:787, 1914-1915.

26. The silver carbonate pyridin bath is prepared as follows: silver nitrate, 10 per cent, 30 cc.; lithium carbonate, saturated aqueous solution, 30 cc. Wash precipitate several times in double-distilled water, decanting and discarding the washings. When the precipitate is thoroughly washed, add 100 cc. of double-distilled water, and dissolve from one-half to three-fourths of it with ammonia water added drop by drop. Filter the supernatant fluid into an opaque bottle and store in dark. This stock solution of silver carbonate may be kept for from two to four weeks.

The bath is made as follows: silver carbonate, 5 cc.; distilled water, 5 cc.; pyridin, 2 or 3 drops.

The porcelain dish in which the sections are heated should not be washed after use, as the silver coating improves the impregnation.

The bile canaliculi are clearly outlined in dark brown, the cell nuclei and reticulum stained deep brown to black, and the cytoplasm golden yellow. The outstanding feature of the method is the clarity of the histologic detail even under the highest magnification. It is such that satisfactory photomicrography can be easily carried out, a desideratum which before has rarely been attained.

### THE DISTRIBUTION AND GENERAL CHARACTERISTICS OF THE BILE CANALICULI

With this method, it is at once apparent that the intercellular canaliculi lie entirely within the cords of hepatic cells so that a portion of the cytoplasm of the cells always intervenes between the canal, on the one hand, and the adjacent sinusoid with its perivascular reticular meshwork, on the other. For this reason it is necessary to have the cell outlines clearly defined and to understand the arrangement of the hepatic cords with regard to the central vein and portal spaces in order to follow the course of the intercellular bile passages. A correlation between the visible canaliculi and the hepatic columns enables one to do this with comparative ease, especially as in these preparations the reticular fibers forming the parenchymal stroma are strongly in evidence.

In sections which have passed exactly transversely or longitudinally through the mantle of secretory tissue surrounding the hepatic veins, it is clear that more of the cords are radially arranged than are irregularly anastomotic. In the same way the canaliculi which form the axes of the cords, and which for that reason may be called axial canaliculi, form a network the components of which are more radial as regards the central vein and portal spaces than anastomotic between the canaliculi of neighboring columns (figs. 1, 2 and 3). This indicates that the flow of bile is from the central portion of the lobule to the periphery where the terminal bile ducts are situated. In the immediate vicinity of the central veins and at those portions of the cell mass where two or three lobules coalesce (the nodal points of Mall [27]), the hepatic columns are irregularly anastomotic from side to side both in the transverse section and from above downward parallel to the portal spaces. They are not here predominantly directed toward any one single point. The canaliculi form a correspondingly tangled network from which the bile may flow in a variety of directions but must eventually find its way into the axial or radial canaliculi directed toward the nearest portal space (fig. 1). All the tiny channels which drain individual cells of the cords and transfer their contents to the axial canaliculi, either of the radial or more irregular type, make

27. Mall, F. P.: A Study of the Structural Unit of the Liver, Am. J. Anat. **5**: 227, 1906.

up the collateral system. Around the portal spaces and in addition to the simple axial tubules with their collateral branches, one finds slightly larger channels usually situated where the columns have fused to form sheets of secretory tissue. These canaliculi receive tributaries both from the axial and the collateral tubules and are referred to by Letulle as collecting canaliculi (fig. 2).

For purposes of description one may therefore recognize three components of the canalicular network, the collateral system, the axial

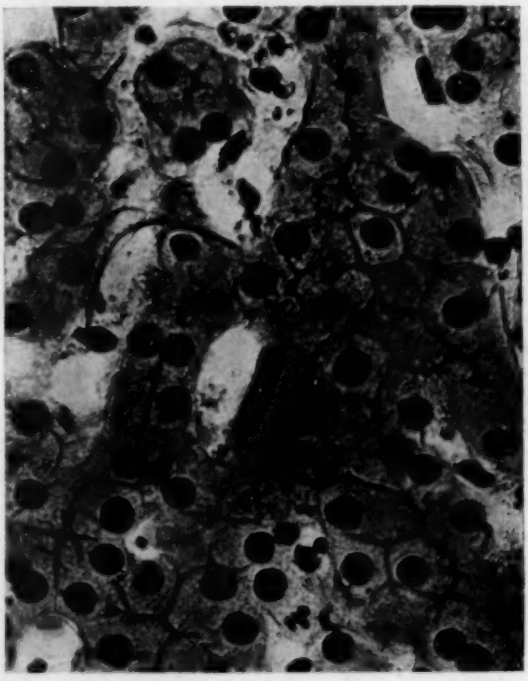

Fig. 1.—Portal canal below, central vein above (outside the boundaries of the illustration). A number of simple radial cords have fused to form a sheet of hepatic cells. The canaliculi become more complicated from above down, all varieties of branches being visible. Axial canaliculi with many collaterals open into collecting tubules at lower left and lower right corners. Many cells are completely surrounded by canaliculi of collateral network; × 650.

canaliculi, and the collecting canaliculi, in addition to the canals of Hering at the junction with the bile-duct system.

The passage of bile from the hepatic cell to the periphery may be briefly traced in the following way. Commencing with the tenuous collateral intercellular canaliculus as the earliest and smallest representative of the intercellular system, the bile passes to the axial canaliculus of the hepatic column, each of whose individual cells is drained by one or

more tubules of the collateral network. The axial canaliculus may pass directly to the bile duct; or if it lies within a more complicated type of cell column, it empties into the slightly larger collecting tubule, from which connections are then made with the bile ducts through the medium of the canals of Hering. The bile ducts themselves, distinguished by their typical lining epithelium, form a many-branched system in the portal spaces, descending through four to six orders which decrease in number but increase in size until the common duct is reached.[28]

The number of times the intercellular system branches, from the tiniest collateral ramification to the union of the axial and collecting

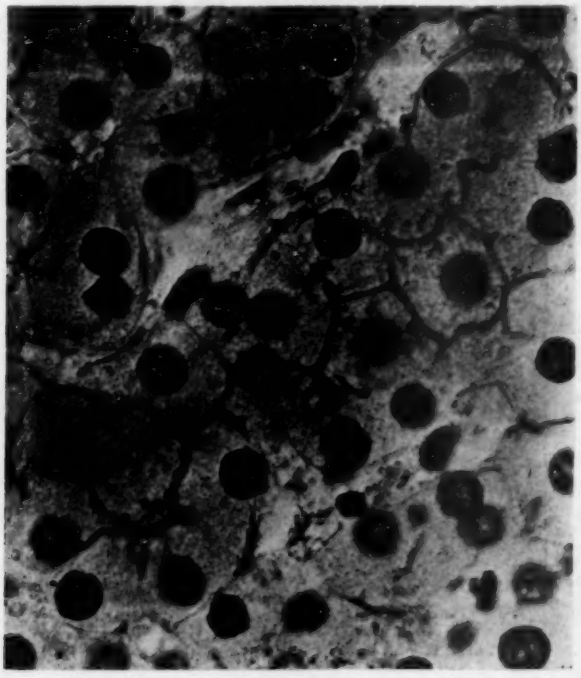

Fig. 2.—Portal canal lower left corner, central vein upper right corner (beyond the limits of the illustration). An axial canaliculus of zigzag type, with blindly ending and anastomotic collateral branches, opens into a collecting tubule of Letulle; × 700.

canaliculi with the bile ducts, varies greatly, and depends entirely on the complexity of the cell columns within which they lie. Sometimes one and sometimes three or four orders of branching may be seen. An examination of their general relationship to the so-called hepatic lobule

28. Counseller, V. S., and McIndoe, A. H.: Dilatation of the Bile Ducts (Hydrohepatosis), Surg. Gynec. Obst. **43**: 729, 1926.

also convinces me that the canaliculi are in no sense divided into a series of anatomically separate areas, but form a vast anastomotic network limited and confined only by the capsule of the liver and to a lesser extent by the separation of the true right and left hepatic lobes.[29] It is for this reason that interruption in the continuity of large numbers of small bile ducts is without effect on the canaliculi above the obstruction. The insufficiency of the anastomosis across the embryologic median plane is emphasized by the fact that the obstruction of either the right or left hepatic duct is followed by obstructive biliary cirrhosis of the corresponding half of the liver.

Fig. 3.—Bile ducts in portal canal at lower left corner, central vein above (beyond limits of the illustration). Two angulated axial canaliculi open directly into terminal bile ducts. Note the relationship of the canaliculi to the sinusoids. The intracytoplasmic argentophil granules lie close to the canaliculi but are not connected with them.

## THE STRUCTURE OF THE CANALICULI AND THEIR INTIMATE RELATIONSHIPS TO THE HEPATIC CELLS

Since in any section the canaliculi may be observed either in cross-section or lying longitudinally within the hepatic columns, it is possible

29. McIndoe, A. H., and Counseller, V. S.: The Bilaterality of the Liver, Arch. Surg. **15**:589 (Oct.) 1927.

to examine their walls and to determine their relationships to the hepatic cells. Between the surfaces of any two cells which are in direct contact without the intervention of either sinusoid or reticulum, the canaliculus appears in cross-sections as a tiny hollow tubule from 0.5 millimicron to 1 millimicron in diameter, situated midway between the cell margins which border the adjacent sinusoids (in the simplest form of column). It forms a semicircular indentation on each opposing surface as if a groove had been scooped from it. The tubules are not

Fig. 4.—Cross section of a canaliculus lying midway between adjacent sinusoids. The opposing cell surfaces form a single faint line continuous with the wall of the canaliculus. Double contours are absent; × 1,800.

composed of the unaltered membranes of the cells themselves by mere apposition of these lateral indentations, for, apart from their staining reactions, they showed no evidence of being formed of lateral halves. Nor do they possess a separate membranous wall, as has been frequently stated, for double contours are entirely absent. The silver impregnation is most intense in the inner surface of the tubule whence it rapidly shades off until it is lost in the cytoplasm of both hepatic cells. The opposing cell membranes on each side of this circular structure appear as a single faint brown line which blends insensibly with the canalicular wall.

The wall of the tubule, therefore, probably represents a modification of the cell membrane itself and as such must be regarded as an integral part of it rather than as a separate membrana propria (fig. 4).

With regard to the arrangement of the canaliculi and the hepatic cells, a regular plan is strictly observed. At the outset, it may be said that in the normal liver, with this method and with a magnification as high as 1,800 diameters, not a trace of intracellular canaliculi, or of any connections or formed structures between the intraprotoplasmic vacuoles of von Kupffer and the intercellular tubules has been observed (fig. 5). While it may be argued that these structures are invisible to

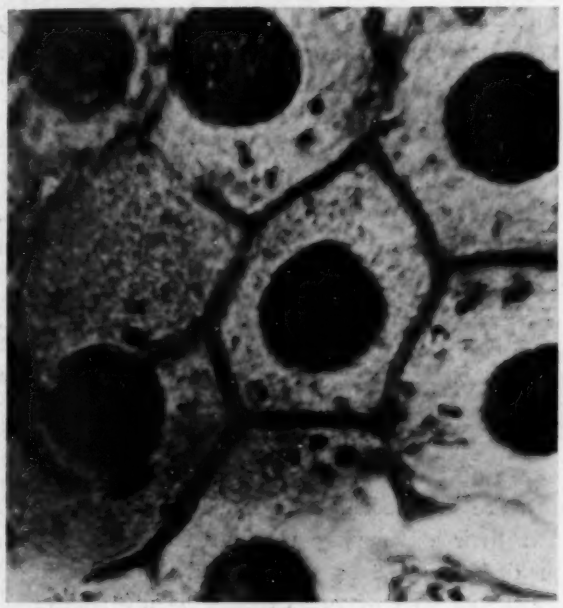

Fig. 5.—A single hepatic cell from a multicellular column entirely surrounded by canaliculi of the collateral network. Intracellular canaliculi are not found. The intraprotoplasmic vacuoles and granules are not connected with the intercellular tubules. The two short branches in the lower right corner are foreshortened canaliculi of the blind type, coursing around the cells; × 1,800.

staining methods, it should be remembered that they were first described in the normal liver by means of a technic much inferior to that of Hortega,[30] the histologic clarity of which is one of its chief recommendations. Certainly a demonstration of their presence by means of injections or by the results of obstruction of the biliary system itself will never be free from the criticism that the phenomenon observed

30. Hortega, Rio, cited by Carleton, H. M.: Histological Technique, New York, Oxford University Press, 1926, p. 267.

represents an eruption of a distinctly abnormal nature. In deciding this matter, it is important not to mistake the identity and position of certain of the blindly ending tubules which on superficial examination appear to extend into the protoplasm of the hepatic cells between those canaliculi which are definitely intercellular. They apparently possess expanded ends and narrow pedicles and are considerably shorter than any of the contiguous intercellular culdesacs. Careful focusing appears to me to show that they actually lie outside the cells but are coursing around them perpendicular to the plane of the section and are thus foreshortened. These structures have probably been frequently misinterpreted as intracellular canaliculi.

Certain intraprotoplasmic granules, presumably secretory products, which, in these preparations appear as multiple discrete argentophil bodies, must also be considered in this connection, for they are gathered especially near the canaliculi. Scanty or entirely absent near the portal spaces, they become exceedingly numerous as the central vein is approached, and here may obscure the details of the canalicular system. While the exact nature of these granules is a matter of speculation and will not be considered here, careful observation convinces me that they are not in any way connected with the intercellular canaliculi and that they do not lie within any definite intracellular ductal apparatus (figs. 3 to 5).

In the simplest form of hepatic column, that consisting of a double row of hepatic cells running straight from the vicinity of the central vein to the portal space without lateral anastomosis, the axial canaliculus, in this case of radial type, lies midway between the surfaces of the cells which are in direct contact and runs parallel to the adjacent sinusoids with their enveloping reticulum. Its course is markedly zig-zag, each deflection being slightly curved following the cell it touches, while the number of turns corresponds to the number of cells in the length of the cell column. Frequently it is irregularly undulant rather than angulated. From the convexity of each turn and twist, a short tubule is projected between the opposing surfaces of successive cells on each side and ends abruptly as a culdesac, approximately half way between the axial canaliculus and the sinusoid on the opposite face of the cell (figs. 2 and 3). Sections through the opposite longitudinal plane of the same type of hepatic column will appear as a single row of hepatic cells between parallel sinusoids, the second row now being beneath the visible layer. The collateral branches are now seen in cross-section, as a row of tiny circles each at the mid-point of successive opposing cell surfaces. As the focus approaches the second layer, the axial canaliculus again comes into view. The effect of this arrangement is that the collateral canaliculus is usually separated from the

adjacent sinusoid by the width of at least half a hepatic cell, while the axial canaliculus maintains a distance of half a hepatic cell in one plane and a whole cell in the other.

Where the cords form complicated turns and anastomose frequently from side to side, or by fusion produce sheets of parenchyma, the disposition of the canaliculi is not so simple, although their general relationship to the vascular system is still maintained. Here the relatively simple series of culdesacs becomes a network of collateral tubules in close contact with the cell mass, and, in many instances, by multiple anastomosis produces a mosaic of polygonal spaces each occupied by a single hepatic cell (fig. 5). In such cases, canaliculi are in contact with the angles of the cell as well as with the surfaces. Blindly ending branches are rarely seen, for those which at first sight appear to form culdesacs, on further examination are seen to turn sharply upward or downward to communicate obviously with cords of cells not included in the section, their ends being cut transversely. Rarely can more than one axial canaliculus be found on one pair of cell surfaces, but every hepatic cell is in contact with one or more representatives of the collateral system, depending on the number of cell faces not in contact with vascular channels. In these situations, too, the slightly larger channels or collecting canaliculi of Lettule, formed by the union of many collateral branches or by the junction of two or more axial canaliculi, can be distinguished. They are seen at many points in the trabecular mass but are commonly found where the columns are large and their cells most numerous. For this reason, they are more frequent in the peripheral areas of the lobule, and on cross-section lie in the centers of circular groups of cells having the appearance of typical gland acini (fig. 6). As the points of confluence of rosets of collateral canaliculi, both of the anastomotic and blind type, together with the axial canaliculi of longer columns of cells, they represent a more complicated type of the simplest form of tubule already described, owing to the fact that they drain larger areas of secreting tissue. While the collecting tubules may be said to have both the collateral and the axial canaliculi as tributaries, the latter do not necessarily open into them but may pass directly to the bile ducts when the cords within which they lie abut directly on the portal spaces (fig. 3).

### THE CANALS OF HERING

A canal of Hering, far from being an actual canal, is the point at which the lumen of the finest bile duct is continuous with the lumen of any member of the canalicular system. There can be little doubt that it exists, although it is much the most difficult part of the excretory pathway to demonstrate histologically. As Beale says, it represents the

narrowest and most vulnerable part of the excretory ducts and is broken by the slightest trauma. For this reason, it is necessary to ensure that the tissue be fresh before fixation, the blocks cut with a sharp thin-bladed knife, and the sections treated with the utmost care during the process of impregnation. The smaller bile ducts within the portal spaces are easily recognized by their deeply argentophil nuclei and faintly refractile cytoplasm lying on a basement membrane which is surrounded externally by peribiliary reticulum, similar to that around the blood vessels. At the extreme edge of the portal canals, tiny,

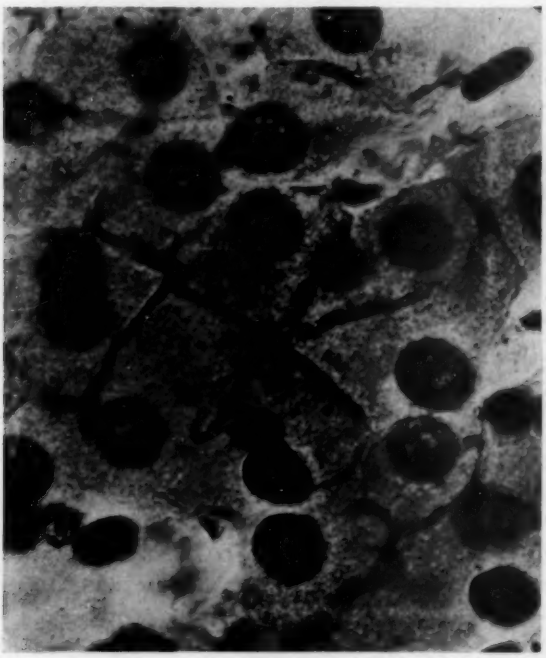

Fig. 6.—A collecting tubule of Letulle in cross section. It receives collateral and axial canaliculi and lies within a typical acinus of hepatic cells; × 1,000.

abruptly ending branches are given off which penetrate the hepatic cell mass for a distance of two, three, four or more cubical epithelial cells. The cytoplasm of the cellular lining of these terminal ducts stains poorly although it can be made more prominent by the use of a 1 per cent solution of eosin following impregnation. The basement membrane persists, but very little reticulum is to be seen, since it becomes continuous with the "gitterfasrn" of the intercellular sinusoids in common with the remaining periportal connective tissue. This dispersal of fibers takes place from the very edge of the portal canals. In favorable specimens careful search under oil immersion shows

excellent examples of the intact junctions between the columns of hepatic cells and the bile ducts, though many more are obviously dissociated. There are in general two kinds of entrance of the canaliculi into the ducts. In the first, there is a sudden transition from the cubital epithelium of the bile duct to the polygonal cells of the hepatic column directly apposed to its tip (fig. 7). Such endings are most easily found at the angles of the portal canals where the connective tissue spreads out in a fan-shaped manner, defining more clearly than elsewhere the terminal ducts and purely radial columns. I was unable

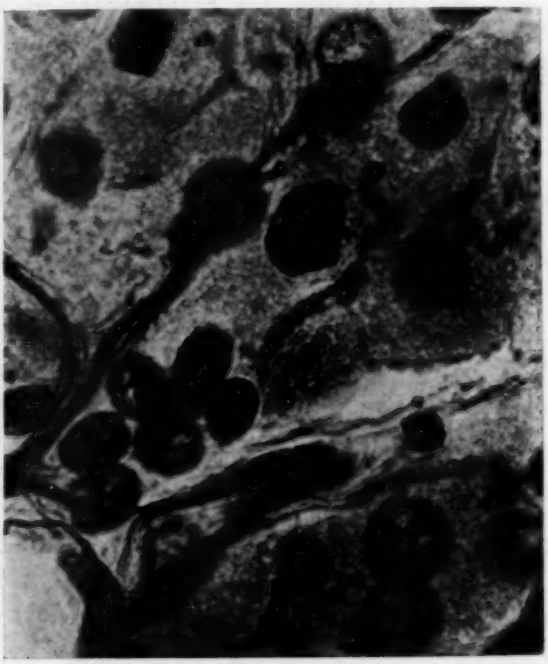

Fig. 7.—Portal canal in lower left corner. A canal of Hering showing the entrance of the canaliculus into the terminal bile duct. Note the tiny ampulla with a flangelike tip at the mouth and the absence of transitional cells and peribiliary reticulum; × 1,100.

to confirm the finding of Letulle that a transitional type of epithelium exists or that the passage from one to the other is a gradual one. The canaliculus, either of the simple axial type, or derived from the collecting tubule, runs straight to the mouth of the duct and there forms a tiny ampulla by a very slight widening out of its terminal portion. Sometimes two or more canaliculi unite to form a single ampulla. This widening, however, is not constant. The modification of the canalicular wall appears to extend for a short distance onto the ductal surfaces

of the last two cord cells, forming a kind of flange at the mouth and is then lost in the basement membrane of the duct directly apposed to it. The second variety consists in the abutment of the cell columns on the lateral walls of the same kind of fine terminal duct with little or no enveloping reticulum, at which point the canalicular walls again become lost in the basement membrane and the lumen is continued between the epithelial cells of the duct wall (fig. 8). An ampullar union is here more uncommon, although the same sudden transition between the cells is still evident.

Fig. 8.—Portal canal below; two canaliculi entering a bile duct laterally. Diameter of canaliculus slightly greater than that of collateral branches and equal to that of axial canaliculus. Transitional cells are not present; × 1,200.

There are certain points of interest with regard to both types of junction. The diameter of the last portion of the canaliculus before the ampulla is formed is usually less than it is elsewhere. Although an axial canaliculus may, if it be of medium size, unite with a bile duct without appreciable decrease in its diameter, the collecting tubule appears to become definitely narrower until it once more approximates the size of the axial canaliculus. In other cases, narrow branches arise at right angles from the collecting tubules and after a short course empty into the ducts (fig. 9). A few specimens showed a direct communication without decrease in the lumen, but such a mode of entrance

was rare in comparison with the former methods. Thus the diameters of most of the canaliculi which enter bile ducts are, at the junctional points, approximately the same, and equal to that of axial canaliculus of medium size.[31] It is further noticeable that considering the huge number of canaliculi, the number of canals of Hering is comparatively small. The canalicular system by reason of its rich intercellular anastomosis necessitates drainage only at certain points along the course of the portal canals, and for the same reason can tolerate interruption in the continuity of many of these drainage points without ill effects.

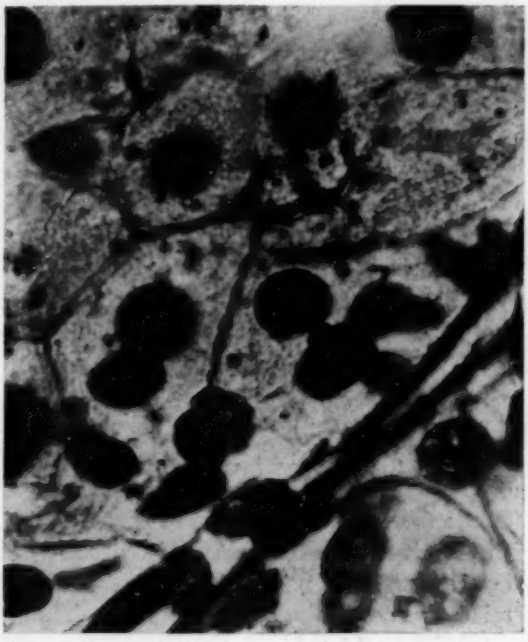

Fig. 9.—Portal canal in lower right corner. Small branch from collecting tubule entering bile duct at edge of portal space; × 1,000.

## SUMMARY

By means of the Rio Hortega silver carbonate method, the bile canaliculi of the liver of man are demonstrated with great clarity and

31. One cannot distinguish even at this point any sign of a distinct membranous wall bridging the gap between the two structures such as that described by Eberth and Milne. The wall of the canaliculus being a modification of the cell membrane and an integral part of it therefore never appears prolonged beyond the end of the cell column and, where separation of the two has occurred, lies open at the end of the broken cord but flush with its surface. In whatever way the hepatic columns are joined to the bile ducts the union is exceedingly fragile.

precision. They exist as an intricate system of intercellular tubules anastomosis of which is limited only by the capsule of the liver and to a lesser extent by the separation of the true right and left lobes of the liver. The canaliculi of adjacent hepatic lobules are in free communication. Three types of branches are to be distinguished, depending on the complexity of the cell columns within which they lie. The simplest form is a short culdesac projected between the contiguous surfaces of two hepatic cells and ending halfway between the parent canaliculus and the adjacent sinusoid. It is most commonly found in the simplest form of cell column. With it are associated fine anastomotic tubules of the same size which drain outlying cells of the more complicated cords and occasionally entirely surround the cells as a mosaic of tiny bile passages. Both of these make up the collateral system of canaliculi. The second variety is the axial canaliculus, which forms the axis of the cell column and receives the collateral branches of the previous group. Certain of them are radially arranged, and certain others irregularly anastomose between themselves according to the arrangement of the cell columns within which they lie. They may empty into the bile ducts directly, or, if situated in sheets of hepatic cells formed by fusion of many cell cords, open into larger so-called collecting tubules, forming the third component of the fine excretory system. Connections are then made with the bile ducts in the portal spaces either directly or by fine lateral branches. Intracellular canaliculi could not be demonstrated. The connections between the bile canaliculi and bile ducts in the portal spaces are difficult to show because of their extreme delicacy and tendency to rupture. Two varieties are found: first, a direct apposition of the end of a cell cord and the tip of a bile duct with continuity of the two lumina and with no transition between the polygonal cells and the biliary epithelium; second, a lateral entrance of the canaliculi through the walls of the very finest ducts which are devoid of reticulum. An ampulla is sometimes present at the site of union and may receive more than one canaliculus. Transitional cells are also absent here. The canaliculi are structurally a modification of the membranes of the hepatic cells and an integral part of them. The relative fewness of the canals of Hering is related to the free canalicular anastomosis and this to the gross obstruction which must occur before jaundice becomes clinically evident.

# THE TRANSFER OF IRRITATED OMENTUMS WITH SUBSEQUENT IMMUNITY TO STREPTOCOCCI *

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The work reported in the present paper represents an extension of the technic of cellular transfer, and, more particularly, of clasmatocyte transfer. This technic has been the subject of several reports.[1] One may sum up the conclusions of these papers which bear on the present experiments briefly by saying that areas of granulation tissue in the walls of one pleural cavity protect against streptococcus infection in the other cavity; similarly that such granulating areas in the peritoneal cavity protect against streptococcus infections in the pleural cavity. In the latter case, which has been studied in detail, an apparent migration of clasmatocytes from the peritoneal to the pleural cavity was observed, and it was further found that animals without such peritoneal granulation tissue did not show any such migration and were not protected. The protection was present in 75 per cent of the animals studied; the number of organisms varied between 100 and 250 times the minimum lethal dose.

The close relationship which has been established between clasmatocytes and intrapleural streptococcus infections in rabbits has been described in several papers by Gay and his co-workers [2] and need not be repeated here.

It has been the purpose of the present experiments to transfer an area containing large numbers of clasmatocytes into the peritoneum of a normal animal and then to test any possible immunity by intrapleural inoculation with *Streptococcus hemolyticus*. The area chosen for transfer was the omentum, since many previous observations have shown that this tissue responds quickly to irritation with a large increase in its cell content, and specifically in the number of clasmatocytes present. Here, as in previous work, rabbits were used.

The literature on omentum transfer is extremely meager; in fact, none of it bears directly on the present experiments. Le Play [3] found

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* From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

1. Gay, F. P.; Linton, R. W., and Clark, A. R.: Proc. Soc. Exper. Biol. & Med. **24**:23, 1926. Linton, R. W.: Mobilization and Transfer of Clasmatocytes, Arch. Path. **5**:787 (May) 1928; Proc. Soc. Exper. Biol. & Med. **35**:288, 1928.

2. Gay, F. P., and Morrison, L. F.: J. Infect. Dis. **33**:338, 1923. Gay, F. P., and Linton, R. W.: Proc. Soc. Exper. Biol. & Med. **23**:325, 1926. Gay, F. P.; Clark, A. R., and Linton, R. W.: Histologic Basis for Local Resistance and Immunity to Streptococcus, Arch. Path. **1**:857 (June) 1926.

3. LePlay, A.: Compt. rend. Soc. de biol. **71**:484, 1911.

that resected omentums left in the peritoneal cavity retained the power to react to foreign bodies in exactly the same way as normal omentums. Histologically the tissue was of normal appearance after six weeks. Muller [4] transferred omentums from guinea-pigs immunized against sheep erythrocytes to normal guinea-pigs, and observed that the latter showed antibodies against the sheep cells in their blood. Antibodies also appeared after the transfer of spleen and bone marrow. This author believed that there is a true secretion of antibodies by the transplanted organs, rather than a resorption of antibodies which were preexisting in the transported tissue. Portis [5] transferred the omentums of normal rabbits to omentectomized controls and found that these animals produced the same amount of antibodies as rabbits without omentums, namely, one quarter of the amount found in normal rabbits. When it was studied histologically, two weeks after its insertion, the omentum had become aplastic.

There is little agreement as to the fate of the omentum either resected and left in its own peritoneum or transferred to another cavity. Rubin [6] stated that a detached piece of omentum in the peritoneum rapidly becomes necrotic. LePlay,[3] on the other hand, found the detached omentum fully viable after six weeks, and showing normal blood vessels and endothelial cells. Several workers have shown that omental grafts will survive. Thus, Davis [7] inserted bits of omentum in the liver and spleen and showed that after weeks and months they were still viable. Finton and Peet,[8] working on dogs, found that free omental grafts survive for at least six months practically unchanged. Survival was found also by Brocq, Ducastaign and Reilly.[9] These workers showed that the characteristic morphology of the omentum was unchanged after long periods.

## EXPERIMENTS

The increase in the cellular content of the omentum was accomplished by the intraperitoneal injection of 10 cc. of a mixture of 5 per cent aleuronat and 3 per cent starch on each of two alternate days. Four days after the second injection the animal was killed; its omentum was removed in several pieces, and placed free in the peritoneal cavity of a normal animal under complete anesthesia. The number of cells in an omentum of this type is much greater than normal The increase is due principally to the clasmatocytes which are present in enormous numbers, and to the number of fibroblasts which is also greatly increased. No such striking changes are found for the other cells; it is notable that there is a complete absence of polymorphonuclears in the tissue itself. The omentum is

4. Muller, L.: Compt. rend. Soc. de biol. **83**:202, 1920.
5. Portis, B.: J. Infect. Dis. **34**:159, 1924.
6. Rubin, I. C.: Surg. Gynec. Obst. **12**:117, 1911.
7. Davis, C. B.: Omental Grafts, J. A. M. A. **68**:705 (March 3) 1917.
8. Finton, W. L., and Peet, M. M.: Surg. Gynec. Obst. **29**:281, 1919.
9. Brocq, P.; Ducastaign, R., and Reilly, J.: J. de chir. **20**:358, 1922.

about twice its normal size. Small lumps of the irritant were adherent to it in most cases. These were removed in each instance before the omentum was inserted in the normal animal.

Four days after the insertion of the omentum, the animals received a dose of *Streptococcus hemolyticus* intrapleurally in the right cavity. The dose varied from one group of animals to another between 15 and 110 times the minimum lethal dose. Altogether, nineteen rabbits were prepared and infected in this manner. Fourteen of these, or 73 per cent, survived or were sterile when killed, while the other five died with the typical empyema caused by the organism (Gay and Morrison).[2] Four of the five animals which died had received slightly more than 100 times the minimum lethal dose, and three others used to test the limit of the protection died after being given 200 times the minimum lethal dose. The protection, then, exists only when 100 times the minimum lethal dose or less has been given.

Three types of controls were used. The first were normal animals given intrapleural doses of 25 and 35 times the minimum lethal dose. These animals died with a typical syndrome. The second set of controls consisted of four animals which had received the omentum of normal rabbits and were given an infection four days later. Three of these which had received doses varying from 20 to 100 times the minimum lethal dose died with a typical picture of empyema. The fourth, which received 45 times the minimum lethal dose was sterile when killed seventy-two hours after infection.

The third control was designed to test whether the protection was due to the irritating effect of the omentum acting as a foreign body, or to the large number of clasmatocytes which had been added to the infected animal. It was thought that the inserted omentum together with the trauma and subsequent granulation at the point of incision might together play the part of an irritant and thus account for the protection. The control consisted of the insertion of omentums from one normal and two irritated peritoneums into three normal rabbits. Before insertion, however, the tissues of these omentums were killed by being placed in water near the boiling point for a minute. These tissues were presumed to act entirely as foreign bodies, and thus the effect of the living clasmatocytes on the infection was ruled out. Four days later the animals were given small doses (fifty times the minimum lethal dose) of streptococcus intrapleurally. Two of them died with typical symptoms. The third, which had received a devitalized irritated omentum, was sterile when killed eleven days later. The possible significance of the survival of this control animal and the one mentioned before will be discussed later. It should be noted, that both animals developed large subcutaneous abscesses around the point of peritoneal incision, whereas the other control animals and the experimental animals showed clean wounds.

Several prepared animals were killed at intervals after they had received the intrapleural dose of streptococcus, and studies were made on the cellular response in the pleural cavities and in the peritoneum. The results are shown in table 1. At the end of six hours the number of organisms present was about thirteen times the number injected. At all the other intervals the animals were sterile. After an initial drop, the proportion of clasmatocytes to polymorphonuclears rose steadily, until eight and eleven days later when they were higher than they were in the normal cavity. This type of cellular response is characteristic of streptococcus infections which are being successfully treated.[10] The proportion as well as the number of polymophonuclears drops steadily after twelve hours. The left cavity and peritoneal cavity do not show any significant changes.

10. Linton (footnote 1, second reference). Gay (footnote 2, first reference).

TABLE 1.—*Study of the Exudates of the Serous Cavities of Rabbits Infected with Streptococcus Hemolyticus Four Days After the Administration of an Irritated Omentum Intraperitoneally*

| | | | | Right Cavity | | | | | Peritoneal Cavity | | | Left Cavity | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | Killed | Dose | Amount of Fluid | Streptococci in Cavity | Clasmatocytes, Cc. | Per Cent Clasmatocytes | Polymorphonuclears, Cc. | Per Cent Polymorphonuclears | Per Cent Clasmatocytes | Per Cent Polymorphonuclears | Streptococci | Per Cent Clasmatocytes | Per Cent Polymorphonuclears | Streptococci |
| Normal..... | | | 0.1 | ..... | 350,000 | 85 | 50,000 | 15 | | | | | | |
| 1 | 6 hrs. | 1,500 | 2.0 | 20,000 | 2,000,000 | 18 | 9,000,000 | 82 | 70 | 30 | Sterile | 80 | 20 | Sterile |
| 2 | 12 hrs. | 1,000 | 0.01 | Sterile | 2,656,000 | 16 | 13,000,000 | 84 | 90 | 10 | Sterile | 54 | 46 | Sterile |
| 3 | 12 hrs. | 820 | 2.0 | Sterile | 13,800,000 | 46 | 16,200,000 | 54 | 70 | 30 | Sterile | 66 | 34 | Sterile |
| 4 | 18 hrs. | 2,000 | 0.1 | Sterile | 1,600,000 | 80 | 400,000 | 20 | 66 | 34 | Sterile | 80 | 20 | Sterile |
| 5 | 24 hrs. | 500 | 0.1 | Sterile | 3,700,000 | 86 | 616,000 | 14 | 65 | 35 | Sterile | 90 | 10 | Sterile |
| 6 | 24 hrs. | 500 | Trace | Sterile | 938,000 | 67 | 462,000 | 33 | 97 | 3 | Sterile | .. | .. | Sterile |
| 7 | 8 days | 560 | Trace | Sterile | ..... | 98 | ..... | 2 | 85 | 15 | Sterile | 99 | 1 | Sterile |
| 8 | 11 days | 380 | Trace | Sterile | ..... | 92 | ..... | 8 | 86 | 14 | Sterile | 88 | 12 | Sterile |

TABLE 2.—*Study of the Exudates of the Serous Cavities of Rabbits Infected with Streptococcus Hemolyticus Four Days After the Administration of a Normal Omentum Intraperitoneally*

| | | | | Right Cavity | | | | | Peritoneal Cavity | | | Left Cavity | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | Killed | Dose | Amount of Fluid | Streptococci in Cavity | Clasmatocytes, Cc. | Per Cent Clasmatocytes | Polymorphonuclears, Cc. | Per Cent Polymorphonuclears | Per Cent Clasmatocytes | Per Cent Polymorphonuclears | Streptococci | Per Cent Clasmatocytes | Per Cent Polymorphonuclears | Streptococci |
| 1 | 24 hrs. | 1,500 | 0.01 | Streptococci + | 1,200,000 | 43 | 1,700,000 | 57 | 50 | 50 | Streptococci + | 100 | 0 | Streptococci + |
| 2 | 48 hrs. | 775 | 0.1 | Streptococci + | 2,940,000 | 35 | 5,400,000 | 65 | 58 | 42 | Sterile | 64 | 32 | Streptococci + |
| 3* | 72 hrs. | 775 | 13.0 | Streptococci + | ..... | 1 | ..... | 99 | .. | .. | Streptococci + | 1 | 99 | Streptococci + |

* Died after 72 hours.

In contrast to other methods which have been used in the treatment of empyema due to streptococci (granulation tissue in the pleural wall; granulation tissue in the peritoneum), this method of omental insertion gives a much quicker clearing up of the infection, which, in turn, is coincident with the more rapid appearance of clasmatocytes in the pleural cavity. For example, with areas of granulation tissue in the peritoneum, the exudate in the right pleural cavity shows only 8 per cent clasmatocytes twenty-four hours after infection, in contrast to the average of 75 per cent in the experiments in which the omentum was tranferred. The first type of cavity shows 40,000 times the number of organisms injected, in contrast to the "omentum transfer" animals, the cavities of which were sterile.[11]

Furthermore, Gay, Clark and Linton [12] found that in areas of granulation tissue formed directly in the pleural cavity, the clasmatocytes present twenty-four hours after infection amounted to but 20 per cent of the total number of cells, although the absolute number of clasmatocytes was much greater than that in the present experiments. The response of the "omentum transfer" animals is thus quicker as to the relative proportion of clasmatocytes which appear in the infected cavities. Furthermore, as Gay [12] has pointed out, the relative proportion of these cells, rather than their absolute numbers, is the deciding factor in the outcome of streptococcus infections of this type.

Table 2 gives data on the reaction of animals which received a normal omentum in place of an irritated one. These animals were studied one, two and three days after infection. The cellular relationships were in complete contrast to those found in the protected animals. The polymorphonuclears increased rapidly in proportion, until at death they numbered more than 99 per cent of the cells present in the exudates. On the other hand, the clasmatocytes decreased until they had almost disappeared. A similar change took place in the left cavity and, to less extent, in the peritoneum. It should be added that the control animals die in three days only when the infection is particularly acute. More usually death occurs between the fifth and sixth days. The cellular picture is the same, however, as in the acute conditions.

### HISTOLOGY

Microscopically none of the inserted omentum showed differences corresponding to the different intervals during which they had been in the foreign peritoneum. Nor could the irritated omentum be distinguished from the normal inserted ones, but all showed much the same structure. They had become almost acellular, and only strands of connective tissue marked out the former structure. Granulation tissue of varying thickness surrounded the omentum, and was infiltrat-

11. Linton (footnote 1, second reference).

12. Gay, Clark and Linton (footnote 2, third reference).

ing the small necrotic areas which were usually present near their margin. It was clear that the irritated omentum, in becoming acellular and indistinguishable from the normal inserted omentum, had given up a much larger number of clasmatocytes.

The histologic changes in the infected cavities were not marked and may be described briefly. Six hours: The tissues surrounding the right pleural cavity did not show any striking changes. The parietal pleura was covered with a slight serous exudate in which red blood cells were numerous. These cells were also numerous in patches under the serosa. Polymorphonuclears were rare, while the number of mononuclears was definitely increased. Twelve hours: The histologic condition here was hardly abnormal. The serosal cells of the parietal pleura were swollen, and the number of clasmatocytes subserosally was increased. On the diaphragm the subserous lymph spaces were swollen. Organisms could not be found. Eighteen hours: Here again the histologic picture was one of slight change. There was not any exudate or infiltration of polymorphonuclears. As before, the infiltration was mononuclear. Throughout the diaphragm there was an intramuscular infiltration of mononuclears. Twenty-four hours: The condition was similar to that in the twelve and eighteen hour animals, with the exception of the diaphragm of one of the animals. Throughout this diaphragm there was an enormous infiltration of mononuclears, with a considerable amount of granulation tissue on both sides, but especially on the peritoneal side. Since this infiltration was so extreme and so much more striking than that in any of the other sections, including those of the other twenty-four hour animals, it was conjectured that it had been caused by the adhesion to the diaphragm of a piece of the inserted omentum. Such an adhesion was observed at autopsy.

The histologic study of animals with normal omentums, killed twenty-four and forty-eight hours after infection did not show any striking changes in the tissues surrounding the pleural cavities. In fact, the only differences between these cavities and those of animals which were eventually protected were that the former had not become sterile and showed a constantly decreasing proportion of clasmatocytes in their exudates. The walls of the unprotected cavities eventually showed an acute inflammatory condition, but it appeared much less rapidly than it did in perfectly normal animals.

It seems probable, therefore, that normal inserted omentum plus the granulation tissue in the peritoneal incision gives some slight protection which may delay the acute phase of the infection for a day or two in some animals. When, in addition, a large abscess develops around the point of incision the protection may be complete, as in the control animals mentioned previously. This condition is equivalent to a "fixation abscess."

Further evidence for this view is found in a comparison of these "normal omentum" rabbits with perfectly normal animals given *Streptococcus hemolyticus* intrapleurally. The number of clasmatocytes decreases much more rapidly in the latter animals, there being only 5 per cent at twenty-four hours and none at forty-eight hours,[13] while the animals on which peritoneal operations were performed showed 43 and

13. Gay (footnote 2, first reference).

35 per cent, respectively, at the same intervals (table 2). That is, the disappearance of these cells is less rapid, and the acute phase of the infection is held in check just this much longer.

The left pleural cavities in the protected animals do not show deviations from the normal either histologically or in their cell counts, and they do not become infected. In the "normal omentum" animals they have become infected at twenty-four hours and remained so until death. The peritoneal cavities of these animals also contain fewer clasmatocytes than are found in the protected animals.

Briefer account may be given of experiments now under way in which it is hoped to establish a therapeutic value for the inserted omentum. In this study the infecting dose is given twenty-four hours before the insertion of the omentum.

TABLE 3.—*Study of Animals Given Irritated and Normal Omentums Intraperitoneally Twenty-Four Hours After Intrapleural Infection with Streptococcus Hemolyticus*

| Animal | Intrapleural Dose M. L. D. | 24 Hours Later Given Intraperitoneally | Result |
|---|---|---|---|
| 1 | 60 | Irritated omentum | Survived |
| 2 | 60 | Irritated omentum | Survived |
| 3 | 60 | Irritated omentum | Survived |
| 4* | 60 | Normal omentum | Died after 4 days; typical empyema |
| 5 | 90 | Irritated omentum | Died after 3 days; typical empyema |
| 6 | 90 | Irritated omentum | Died after 5 days; typical empyema |
| 7 | 90 | Irritated omentum | Died after 2 days; typical empyema |
| 8* | 90 | Normal omentum | Died after 3 days; typical empyema |

* Control.

Eight animals were infected intrapleurally in the right cavity with *Streptococcus hemolyticus*. These were divided into two groups: four had received 60 times the minimum lethal dose and four 90 times the minimum lethal dose. Twenty-four hours later, under local anesthesia, an irritated omentum was placed in the peritoneums of three in each group. The other animals of each group received a normal omentum under the same conditions. This experiment and its results are summarized in table 3.

This preliminary experiment shows that 90 times the minimum lethal dose is beyond the dose which can be tolerated by animals in which an attempt at cure has been made after the infection is under way. On the other hand, the animals given the smaller dose all survived with the exception of the control, which had received an untreated omentum. While the mechanism of recovery under these conditions has not yet been studied in detail, it appears probable that in this case, as in the work reported in the first part of this paper, a clasmatocyte infiltration induced either directly or indirectly by the irritated omentum will be found responsible for the survival of the animals.

SUMMARY

Rabbits which have received irritated rabbit omentums intraperitoneally, and which are subsequently infected intrapleurally with *Streptococcus hemolyticus,* survive the infection in about 70 per cent of the cases.

The survival is correlated with the appearance in the infected cavity of a large proportion of clasmatocytes.

A preliminary experiment shows that survival may occur when the omentum is inserted twenty-four hours after intrapleural infection has begun.

# NEW ASPECTS OF THE FORMATION AND CLASSIFICATION OF GALLSTONES *

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The chemical composition and architecture of gallstones is fairly well known; at least, a classification of gallstones based on such properties is found in every textbook on pathologic anatomy. The formation of gallstones, however, is still one of the many riddles of human pathology. A precise summary of knowledge of the subject is contained in Wells' "Chemical Pathology." [1] Aschoff,[2] during a visit to this country in 1924, discussed Naunyn's and his own views on the origin of gallstones. The fourth Ludvig Hektoen Lecture of the Billings Foundation, delivered by Peyton Rous [3] of the Rockefeller Institute for Medical Research, is entitled: "Physiological Factors in the Genesis of Gallstones." The facts they present adequately testify that knowledge of the cause and the mode of formation of gallstones is rather meager. This, however, is not to be wondered at in view of the fact that we know but little of the function of the gallbladder, which is recognized as the most usual site of the formation of gallstones. Every theory of the formation of gallstones is based on a theory of the function of the gallbladder. The worth of the one determines the correctness of the other.

## CURRENT CONCEPTIONS OF THE FORMATION OF GALLSTONES

Naunyn believed that the cholesterol and the calcium contained in gallstones were secretory products or products of the decomposition of the inflamed mucous membrane of the gallbladder and the biliary ducts. He adhered to these views to the last [4] in spite of the weighty morphologic and experimental evidence produced by Aschoff and his school, proving that the lipoidal substances occasionally found in the lining epithelium and the delicate folds of the mucosa of the gallbladder have been resorbed from the content of the vesicle and are on their way to be returned into the circulation and not to be excreted into the bile.

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* From the Department of Pathology, University of Chicago.

1. Wells, H. Gideon: Chemical Pathology, Philadelphia, W. B. Saunders Company, 1925.

2. Aschoff, Ludwig: Lectures on Pathology, New York, Paul B. Hoeber, Inc., 1924.

3. Rous, Peyton: Physiological Factors in the Genesis of Gallstones, Proc. Inst. Med., Chicago **7**:33, 1928.

4. Naunyn, B.: Zur Lehre vom Aufbau und Umbau der Gallensteine, Mitt. a. d. Grenzgeb. d. Med. u. Chir. **37**:545, 1924.

Inflammation produced by bacterial infection coupled with biliary stasis is, according to Naunyn, the principle cause of the formation of gallstones. Aschoff and Bacmeister denied the infectious origin of the stones composed of pure cholesterol and of pure pigment (calcium bilirubinate). The dependence of the cholesterol content of the bile on the cholesterol content of the blood, definitely proved by animal experimentation, supports this view. From all that is known, it appears quite clear that the stones of pure cholesterol form independently of inflammation, and as a result of a faulty metabolism of cholesterol, an increased output of cholesterol by the liver and a failure of adequate removal of the cholesterol by the mucosa of the gallbladder from the bile pouring into the gallbladder. The origin of the stones of pure pigment (calcium bilirubinate) is not clear. According to Thudichum,[5] Naunyn,[6] Boysen [7] and Rovsing,[8] they are formed in the finer intrahepatic biliary ducts. To quote Aschoff, "it is a question of a dyscrasic stone formation, without being able to say on what this dyscrasia depends."

The other most widely assumed cause of the formation of gallstones is biliary stasis. The peculiar thing about this assumption is that there is no definition of biliary stasis given in either Naunyn's or Aschoff's papers. Yet Aschoff and Bacmeister [9] went so far as to introduce the idea of a "stasis gallbladder" and presented the morphologic characteristics attributed to such a condition of the viscus. In the etiology of their "stasis gallbladder," the most important part is played by conditions hindering free in and out flow—that is, the exchange of bile in the biliary vesicle. Such obstacles, all of a mechanical nature, are the following: ill-adapted articles of clothing, pressure on the gallbladder arising from the uterine enlargement of pregnancy and that brought about by other actively growing intra-abdominal tumors, general enteroptosis in multipara, and kinking of and tension on the cystic duct caused by adhesions after appendicitis or diseases of the female reproductive organs. It must be admitted that the rôle of such factors in causing

5. Thudichum, T. L. W.: Ueber den chemischen Process der Gallensteinkrankheit beim Menschen und in Thieren, Virchows Arch. f. path. Anat. **156**:384, 1899.

6. Naunyn, B.: Weitere Beiträge zur Entstehung und zum Bau der Gallensteine, Mitt. a. d. Grenzgeb. d. Med. u. Chir. **36**:1, 1923.

7. Boysen, T.: Ueber die Structure und die Pathogenese der Gallensteine, Berlin, S. Karger, 1909.

8. Rovsing, Thorkild: Zur Beleuchtung und Wertschätzung Naunyns und anderer Infectionstheorien bezüglich der Pathogenese der Gallensteinkrankheit, Acta chir. Scandinav. **56**:103 and 207, 1923.

9. Aschoff, L., and Bacmeister, A.: Die Cholelithiasis, Jena, Gustav Fischer, 1909.

hindrance of the free flow of bile from the gallbladder is vague and the more so if one considers that under such conditions the flow of bile into the gallbladder must be hindered as well. To attribute any significance to ill-adapted articles of clothing is certainly out of date. It is well, however, to retain the conception of "stasis gallbladder" but in a somewhat modified form.

It should be noted here that in a recently published monograph by Lütkens [10] from Aschoff's laboratory, entitled "Aufbau und Funktion der extrahepatischen Gallenwege," a whole chapter is devoted to the "disturbances of the mechanical functions of the extrahepatic bile ducts," but "stasis gallbladder" is barely mentioned. On the other hand, much stress is laid on stasis in the biliary ducts, "Gallenwegestauung," of which Lütkens distinguishes several types: (1) stasis in all the biliary ducts (a) including the gallbladder and (b) excluding the gallbladder; (2) stasis in a circumscribed, mainly intrahepatic portion of the biliary ducts; (3) stasis in the gallbladder with deficiency of its function; (4) all possible combinations.

John Berg,[11] the Scandinavian surgeon, was the first to point out the necessity of a strict differentiation between a "mechanical biliary stasis"—that is, one due to a mechanical obstruction to the free passage of bile into the duodenum—and a "functional biliary stasis" (mainly in the gallbladder) due rather to functional disturbances than to easily demonstrable anatomic conditions. "Functional biliary stasis" is, of course, another vague term, since its causes are quite unknown. This term is also to be retained but, as will be seen later, in a somewhat modified form.

From all that has been said thus far, it is easily gathered that the current conception of the function of the gallbladder does not permit a clear-cut definition of either "functional biliary stasis" or of "stasis gallbladder," both of which are of fundamental importance in an explanation of the cause and the mode of formation of gallstones.

### A NEW HYPOTHESIS AS TO THE FUNCTION OF THE GALLBLADDER

Early in 1924, after three years' study of the biliary system, I [12] believed that I had sufficient data to justify a new suggestion concerning the function of the gallbladder. My suggestion was radically unlike the current views, but it seemed to solve many of the riddles encountered in the orthology as well as the pathology of the gallbladder.

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10. Lütkens, Ulrich: Aufbau und Funktion der extrahepatischen Gallenwege, Leipzig, F. C. W. Vogel, 1926.

11. Berg, John: Einleitungsvortrag zum Thema "Gallensteinleiden," Arch. f. klin. Chir. **126**:329, 1923.

12. Halpert, Béla: Neue Wege in der Gallenblasenforschung, I and II. Med. Klin. **20**:408, 1924; **20**:1830, 1924.

According to this new hypothesis, the gallbladder is not a reservoir the function of which is the supplying of concentrated bile whenever there is a call for it in the intestine, but an organ the function of which is the returning of important constituents of the bile into the circulation, and by the resorption of bile, relieving and regulating the pressure within the biliary system while the sphincter of the ductus choledochus is closed. In other words, bile which once has entered the gallbladder does not, under ordinary conditions, leave it again through the cystic duct, but is resorbed by the mucous membrane of the biliary vesicle, and the constituents then returned by the way of the veins and lymphatics into the liver and into the general circulation, respectively. The arguments pro and contra such a conception have recently been summarized by Blond [13] (1928). He calls attention to the fact that the interpretation of the results of cholecystography is easier and the observations less contradictory if it is assumed that the bile enters the gallbladder, not to be stored there and in time expelled, but to be resorbed in toto by the gallbladder mucosa. The fact that bile may and does leave the gallbladder occasionally in small quantities does not invalidate the conception. The anatomic arrangement about the neck of the gallbladder and in the cystic duct, regulating, as it does, inflow and hindering or preventing outflow, shows, to be sure, a wide enough range of variations, from perfect competency (especially in children) to total derangement, mainly in conditions of biliary stasis, to account at least in part for the lack of uniformity in the experimental results.

According to Lütkens, the amount of bile which can be expelled by the gallbladder is limited; it equals the difference between the total amount of bile contained in the biliary vesicle at the beginning of the emptying process and the so-called residual bile, the bile which cannot be expelled because of the position and limited contractibility of the viscus. The amount of bile which can be expelled from the gallbladder comprises, according to the estimation of Lütkens, a fourth or a third of the total volume of the relaxed gallbladder; that is, the "residual bile" is from 66.66 to 75 per cent, and the "expellable bile" is from 33.33 to 25 per cent.

If it is assumed that the bile enters the gallbladder, not to be stored there and in time expelled, but to be resorbed in toto by the mucosa of the gallbladder—"biliary stasis," and "stasis gallbladder" may be precisely defined and the formation of gallstones explained in simple terms.

According to this new conception, the mucous membrane of the normal gallbladder is capable of resorbing all the bile constituents. A

13. Blond, Kasper: Eine neue Arbeitshypothese zur Klärung der Gallenwegsprobleme, Arch. f. klin. Chir. **149**:662, 1928.

disturbance of this resorptive function is the factor leading to biliary stasis in the gallbladder. In other words, biliary stasis—that is, the functional type of biliary stasis—is a disharmony between the bile contained in the gallbladder and the resorptive function of the mucosa of the gallbladder. A gallbladder which cannot properly resorb the bile which is poured into it, is a "stasis gallbladder." The site of the process leading to stasis of bile in the biliary vesicle may be, of course, in the gallbladder itself, but it may be also in the liver, or in both the gallbladder and the liver.

If the stasis of bile in the gallbladder is due to disturbed or altered functioning of the liver, one may speak of an exogenous, a hepatogenous, or a secondary stasis of bile in the gallbladder. In such a case the gallbladder may appear grossly as well as microscopically perfectly normal. Occasionally, the observations suggest prolonged overdistention of the viscus by pressure from within, the wall being thinner than usual, the folds more delicate and farther away from one another. In certain cases, the folds of the mucosa appear larger and plumper than usual, the epithelial cells, particularly certain cells of the subepithelial layer, being loaded with anisotropic lipoidal substances.

If the halt of bile in the gallbladder is caused by intrinsic functional disturbances or demonstrable pathologic conditions of the gallbladder itself, with the functioning of the liver more or less unimpaired, one may speak of an autochthonous or a cystogenous, or a primary stasis of bile in the gallbladder. A variety of processes, such as acute, subacute, and chronic inflammations, new growths, and the like, might lead to such a condition of the viscus. The presence of typical and numerous "Rokitansky-Aschoff sinuses," that is, deep outpouchings of the mucosa of the gallbladder, which dip down into the muscularis or extend as finger-like processes through the muscular coat into the perimuscular layer, is frequently conspicuous. Of the structural changes in the wall of the gallbladder, which are usually associated with the presence of typical and numerous "Rokitansky-Aschoff sinuses," the following may be mentioned as the most constant and conspicuous: (*a*) thickening of the wall of the viscus, due to marked hypertrophy of the muscularis and an increase of the connective tissue forming the perimuscularis, and (*b*) at least a slight cellular infiltration of the subepithelial layer, the intermuscular septums, and the perimuscular layer, in the last especially around the fundi of the "Rokitansky-Aschoff sinuses." [14]

Finally, when the stasis of bile in the gallbladder is due to both a disturbed functioning of the liver and a pathologic condition of the gallbladder, one may speak of chronic stasis of bile in the gallbladder.

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14. Halpert, Béla: Morphological Studies on the Gall-Bladder, I and II, Bull. Johns Hopkins Hosp. **40**:390, 1927; **41**:77, 1927.

A NEW CLASSIFICATION OF GALLSTONES

The most striking evidence of the existence of these three types of stasis of bile in the gallbladder is furnished by the concretions usually found in these conditions. In order to correlate the types of biliary stasis in the gallbladder with the type of concretions usually found associated with them, it appeared necessary to classify gallstones in a way somewhat different from the usual. A few facts about gallstones should be mentioned. Wells' statement that concretions are never composed of one substance in a pure form, but usually consist of a mixture of the constituents of the fluid in which they are developed is particularly true of gallstones. The cholesterol and calcium salts of the biliary pigment are probably present, at least in minute quantities, in all gallstones. Inorganic salts of calcium (carbonate and phosphate) are also present, as well as small amounts of fats, soaps, lecithin, mucus and other organic products, and occasionally traces of copper, iron and manganese. Peculiarly enough, the quantity of bile salts, which are among the chief constituents of bile, is extremely minute.

The chemical composition and the architecture of gallstones are the properties on which their classification is usually based. My classification distributes in three groups all the chemical and architectural combinations of gallstones possible and considers their probable origin as well.

Group I is the group of the "pure gallstones," that is, those consisting purely or mainly of one of the stone-forming constituents of the bile, such as (*a*) cholesterol, (*b*) biliary pigments (calcium bilirubinate) and (*c*) calcium carbonate.

Group II is the group of the "mixed gallstones," that is, those consisting purely or mainly of at least two of the constituents of the "pure gallstones." Here belong (*a*) the stones consisting of cholesterol and calcium carbonate, (*b*) the stones consisting of cholesterol and calcium bilirubinate, (*c*) the stones consisting of calcium bilirubinate and calcium carbonate and (*d*) the stones consisting of cholesterol, calicum bilirubinate and calcium carbonate.

Group III is the group of the "combined gallstones," that is, those (*a*) having a nucleus formed by one of the members of group I coated with any one of the members of group II, or (*b*) having a nucleus formed by one of the members of group II coated with any one of the members of group I. In outline, this chemical composition and architecture might be set forth as follows:

(*a*) Group Ia, Ib, Ic forming the nucleus,
Group IIa, IIb, IIc, IId forming the shell;
(*b*) Group IIa, IIb, IIc, IId forming the nucleus,
Group Ia, Ib, Ic forming the shell.

### A NEW EXPLANATION OF THE FORMATION OF GALLSTONES

The most widely assumed cause of the formation of gallstones is biliary stasis, that is, a stagnation of bile in the biliary ducts, particularly in the gallbaldder. It is known, however, from clinical as well as from experimental observations, that the mechanical type of biliary stasis, that is, one produced in a healthy animal by ligation of the common biliary duct, does not result in the formation of biliary calculi. The functional type of biliary stasis, that which presupposes a disturbed functioning of the gallbladder, is the one responsible for the formation of gallstones. Thus, gallstones are usually found in conditions of stasis of bile in the gallbladder, of which I distinguished the three types: hepatogenous, cystogenous and chronic.

In the case of a hepatogenous stasis, I assumed that the stagnation of bile in the biliary vesicle was due to disturbed or altered functioning of the liver. Is there any evidence to support such a conception? In the mucous membrane of the normal human gallbladder lipoidal substances cannot be demonstrated by the usual histologic methods. The presence of lipoidal substances in certain cells of the subepithelial layer is, however, occasionally the only histologic observation in gallbladders which have been removed by clinical operation because of characteristic signs of disease of the gallbladder. A solitary stone composed of pure cholesterol with masses of crystals of cholesterol in rows on the surface ("a coiled" stone of pure cholesterol), is not infrequently found in such gallbladders. Similar observations are occasionally made also at postmortem examinations. It is of interest to recall, here, that epithelial cells of the mucosa of the gallbladder containing lipoidal substances were first seen by Virchow[15] in 1846, and that he regarded these cells later (1857) as being concerned in the intermediate metabolism of fats, considering that a part of the fats of the bile were resorbed by the mucosa of the gallbladder. Thus Virchow concluded about seventy years ago "that the gallbladder is not a mere reservoir but has a further function, for through it fats find an intermediate passage way back into the circulation." A similar conception was advanced in 1924 by Sweet.[16] It appears from morphologic as well as from experimental observations that the mucosa of the normal gallbladder is capable of resorbing the small quantities of cholesterol normally present in the bile, without its becoming visible to the naked eye or demonstrable by present microscopic methods.[17] In cases of marked

15. Virchow, R.: Ueber das Epithel der Gallenblase und über einen intermediären Stoffwechsel des Fettes, Virchows Arch. f. path. Anat. **11**:574, 1857.

16. Sweet, J. E.: The Gall-Bladder; Its Past, Present and Future, Internat. Clinics **1**:187, 1924.

17. A new microchemic test for cholesterol in the tissue was described by William Boyd in his Studies in Gall-Bladder Pathology, published in the British Journal of Surgery (**10**:337, 1923).

cholesterolemia, however, when the cholesterol content of the bile is correspondingly exaggerated on account of an increased output of cholesterol by the liver, the mucosa of the gallbladder becomes, so to speak, saturated with the lipoidal substances which it has resorbed from the bile. In such conditions the cholesterol content of the bile in the gallbladder becomes greater and greater, and while the other constituents of the bile, those holding the cholesterol in solution, are being resorbed, the concentration finally may reach a point at which the cholesterol will crystallize out at the slighest provocation. Stones of pure cholesterol usually occur in normal gallbladders the mucosa of which may show signs of resorption of cholesterol, and this is to be regarded as evidence of the attempt of the mucosa of the gallbladder to remove the excess of cholesterol in the content of the vesica fellea.

It has been mentioned that the stones composed wholly of pigment, that is, those consisting of calcium bilirubinate, are believed to form in the finer intrahepatic biliary ducts. They might be found then, of course, in gallbladders that are shown microscopically to be intact.

The small concretions of calcium carbonate also occur in gallbladders in which pathologic changes are not demonstrable. It is therefore reasonable to assume that some transient abnormal composition of the bile leads to their formation.

Thus, for the formation of all of the "pure gallstones," the liver is probably primarily responsible. The quantity and quality, or both, of the bile may be such that the mucosa of the normal gallbladder cannot handle it properly; that is, the gallbladder itself may be intact, but not able to remove by resorption all that is poured into it with the bile.

The formation of "mixed gallstones" has generally been associated with infection. Infection and inflammation damage the mucosa of the gallbladder so that not all of the constituents of the bile are resorbed. Whatever physicochemical explanation is given for the process of formation of the "mixed gallstones" in inflamed gallbladders, the fact remains that the retention of the stone-forming constituents of the bile is due in the first place to a diseased condition of the gallbladder. Thus in a cystogenous biliary stasis in the gallbladder, "mixed gallstones" are formed. The stagnation of bile in the biliary vesicle is responsible for the formation of the stone and the infection is responsible for the stagnation.

If a hepatogenous stasis of bile in the gallbladder which has led to the formation of "pure gallstones" precedes a cystogenous stasis, stones will form which have a nucleus formed by one of the members of the group of "pure gallstones" and have a shell formed by one of the members of the group of "mixed gallstones." If, on the other hand, a cystogenous stasis in the gallbladder which has led to the formation of mixed gallstones is followed by a hepatogenous stasis, stones will

form which have a nucleus formed by one of the members of the group of "mixed gallstones" and have a shell formed by one of the members of the group of "pure gallstones." Combined gallstones are formed in chronic stasis.

### A MORE PRECISE DEFINITION OF "FUNCTIONAL BILIARY STASIS"

The new theory of the function of the gallbladder permitted a precise definition of "functional biliary stasis" and led to the differentiation of three types of stasis, each of which is accompanied by gallstones: the hepatogenous stasis accompanied by the formation of "pure gallstones," the cystogenous stasis accompanied by the formation of "mixed gallstones," and the chronic stasis of bile in the gallbladder accompanied by the formation of "combined gallstones." The wide range of differences shown in the architecture and chemical composition of gallstones is the result, on the one hand, of changes in the composition of the bile due to excessive excretion by the liver of stone-forming substances, and the result, on the other hand, of a relative or absolute resorptive insufficiency of the mucosa of the gallbladder.

The story of the formation of gallstones is then, briefly, as follows:

A disturbed functioning of the gallbladder or an abnormal composition of the bile, or both, lead to a stagnation of bile in the gallbladder. The result of the functional biliary stasis is the formation of gallstones. The type of the concretion or concretions formed depends on the kind of functional biliary stasis which produced them. In a hepatogenous or exogenous stasis of bile in the gallbladder "pure gallstones" form, in a cystogenous stasis "mixed gallstones" form, and in a chronic stasis "combined gallstones" form.

It has been frequently stated that gallstones can tell the story of the gallbladder; while this may be true, it is equally true that the story of a gallbladder cannot be told unless its contents are known.

# HETEROPHILE ANTIGEN IN HUMAN BLOOD*

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The literature on heterophile antigens and antibodies was reviewed in a paper previously published.[1] In 1913, Friedberger and Schiff[2] reported the production of a hemolysin for sheep blood in rabbits by injections of human blood. The titer for the sheep blood was higher than that for the homologous antigen, but it was still rather low. Not much attention was paid to this observation until, in 1924, Schiff and Adelsberger[3] reported the production of a heterophile immune serum in rabbits with injections of human blood, type A. The hemolysin for sheep blood, so produced, could be removed from the immune serum by absorption with sheep blood, human blood, types A and AB, and with alcoholic extracts of heterophile organs, but not with human blood, types O and B. Not all rabbits immunized with blood, type A, responded with the production of a heterophile serum. The same problem was investigated, from various angles, by Doelter,[4] Landsteiner and van der Scheer,[5] Amzel, Halber and Hirszfeld,[6] and Witebsky.[7]

In the present study, I undertook to investigate the relation between the heterophile antigen present in human blood and the one found in sheep blood.

Human blood, type A, was injected into two rabbits, type B into two, type AB into two and type O into two. Five tenths cubic centimeter of a 10 per cent suspension of washed cells per hundred grams of rabbit's weight was injected intraperitoneally at seven day intervals. Titrations were done before the injections were begun, then following the third, the sixth, and the eighth injections and at the end of the experiment. For the titrations of the hemolysin, 0.5 cc. of the serum was

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* From the Research Institute of Cutaneous Medicine.

1. Davidsohn: Heterophile Antigens and Antibodies, Arch. Path. **4**:776 (Nov.) 1927.

2. Friedberger and Schiff: Berl. klin. Wchnschr. **34**:1557, 1913.

3. Schiff, F., and Adelsberger, L.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **40**:335, 1924. Schiff F.: Klin. Wchnschr. **3**:679, 1924; abstr., J. A. M. A. **82**:1826 (May 31) 1924.

4. Doelter, Werner: Ztschr. f. Immunitätsforsch. u. exper. Therap. **43**:95, 1925.

5. Landsteiner, K., and van der Scheer, J.: J. Exper. Med. **42**:123, 1925.

6. Amzel, R.; Halber, W., and Hirszfeld, L.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **42**:369, 1925. Halber, W., and Hirszfeld, L.: Ibid. **48**:34, 1926.

7. Witebsky, E.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **48**:369, 1926; **49**:1 and 517, 1927.

used in dilutions of from 1:4 upward, 1 cc. of the guinea-pig complement diluted 1:10, and 0.5 cc. of a 2 per cent suspension of washed blood cells. Physiologic sodium chloride solution was added to make a total volume of 3 cc. Controls of both the complement and the blood cells were included. For the titration of the agglutinins, the same quantities were used, with the exception of the complement. The titrations of the hemolysin were read after an incubation for one hour in the water bath at 38 C.; the titrations of the agglutinins were placed in the icebox over night, after the water bath incubation, and read the next morning. The titers recorded signify the highest dilutions at which complete hemolysis occurred; in the titrations of the agglutinins, they signify the highest dilutions at which a distinct macroscopic agglutination was seen.

Very small quantities of natural hemolysin and agglutinin for human blood were found in the preliminary titrations. None of the rabbit serums hemolyzed human blood completely in a dilution of 1:4 (—0.125 cc.). The highest titer of the natural agglutinins was 1:8(—0.0625 cc.). The titer of the natural hemolysin for sheep blood was rather high, while the natural agglutinin for the same blood was absent in dilutions of 1:4.

Rabbits 1 and 3a were immunized with blood, type A. Rabbit 1 showed complete absence of natural hemolysins and agglutinins for human blood before immunization. After the third injection, its serum hemolyzed human blood A in a dilution of 1:24; after the sixth injection, in a dilution of 1:128, and after the eighth injection, in a dilution of 1:512, which was the highest titer reached. The isophile agglutinins developed in parallel manner, but to a higher degree (the highest titer being 1:3072). The titer of the natural hemolysin for sheep blood was 1:24. The heterophile hemolysin for sheep blood started to develop much earlier than the isophile hemolysin for human blood, and reached its peak after the sixth injection (1:512). In this, as in the other experiments, there was an almost complete absence of heterophile agglutinins.

Rabbit 3a, the serum of which did not hemolyze or agglutinate human blood before immunization, showed only a moderate titer for the isophile hemolysin and agglutinin after the eighth injection (1:96). The heterophile hemolysin for sheep blood reached its peak (titer 1:800 —0.00125 cc.) after the third injection. It dropped considerably after the sixth and went up again after the eighth injection (1:384). In this, and in rabbit 1, the heterophile antibodies developed much earlier in the course of immunization than the isophile and tended to drop in the later course, while the titer of the isophile antibodies was still rising. This is a characteristic, well known feature of the heterophile antibodies. The immunization index—that is, the quotient between the highest titer

reached during the immunization and the one found in the preliminary titration—was much higher for the isophile than for the heterophile antibodies.

None of the six rabbits immunized with types AB, B, and O showed an increase of the hemolysin for sheep blood above the normal. Two of them (immunized with blood AB and B) developed lysins and agglutinins of a considerable titer for human blood.

The heterophile immune serums lost their lysin for sheep blood when absorbed with organs containing heterophile antigen, but retained the isophile lysin for human blood. Absorption with nonheterophile organs did not have this effect. This was tested in an experiment as follows: Livers and kidneys of guinea-pigs were ground finely with five times their weight of sodium chloride solution. The suspensions were centrifugalized for five minutes; the supernatant fluid was discarded and replaced by fresh sodium chloride solution. This procedure was repeated three times, until the supernatant fluid was clear. Then the suspension was passed through two layers of gauze. To 1.5 cc. of the suspension, 0.5 cc. of the immune serum was added; the whole was then shaken and left in the icebox over night. Then it was centrifugalized, the clear supernatant fluid was removed, inactivated for fifteen minutes at 55 C., and titrated in decreasing quantities, from 0.3 to 0.025 cc., for lysin for human and for sheep blood. The procedure was repeated with livers and kidneys of rabbits.

The absorption of immune serum 1 with the organs of the guinea-pig removed the lysin for sheep blood to a marked extent (slight hemolysis occurring with 0.2 cc.), leaving the lysin for the human blood more or less intact (complete hemolysis occurring with 0.2 cc.). Absorption with the organs of the rabbit did not influence the lysin for sheep blood (complete hemolysis occurring with 0.025 cc.). The same results were observed when the other four serums were absorbed, two of which had been produced by immunizing rabbits with human blood, type AB, a third, by immunizing with type B, and a fourth by immunizing with type O. As mentioned, these four rabbits did not, as a result of immunization, develop a hemolysin for sheep blood to any greater extent than is normally found in nonimmunized rabbits. The lysin for sheep blood which they contained was the natural hemolysin for sheep blood, which is closely related to, or identical in its qualities with, the heterophile antibodies, as was shown by various other investigators.

It was attempted to liberate some of the hemolysin absorbed by the suspensions of the organs to see whether these suspensions would act differently on human and on sheep blood after they had absorbed the immune serums. The sediments obtained from the absorption of the immune serums were washed with sodium chloride solution three times and then suspended in sodium chloride solution. They were inactivated for five minutes at 55 C., and titrated with 0.5 cc. of a 2 per

cent suspension of blood after the addition of 0.1 cc. of guinea-pig complement.

The suspensions of guinea-pig liver and guinea-pig kidney hemolyzed sheep blood slightly but not human blood. The suspensions of the organs of the rabbit did not show any difference in their actions on the human and on the sheep blood. A plausible explanation is that guinea-pig organs absorbed some antisheep hemolysin, while they absorbed little, if any, of the hemolysin for human blood. The organs of the rabbit absorbed about equal amounts of the lysin for human and for sheep blood.

The immune serums were absorbed with various types of human and with sheep blood. Five-tenths centimeter of washed packed blood cells were added to 1 cc. of the serum diluted 1 : 10, and the whole was shaken and left in the icebox over night. Then it was placed for one hour in the water bath at 38 C.; it was then centrifugalized, the supernatant fluid was separated, and again 0.5 cc. of the cells added, and the whole placed in the icebox over night. Following the night it was placed in the water bath, then centrifugalized, separated, and finally inactivated at 55 C. for ten minutes. The absorption with human blood of any type removed all lysins for human blood. In serum 1, produced by immunization of rabbit 1 with human blood A, and showing a considerable development of heterophile hemolysins, a difference between the action of type A and type B on sheep lysin was observed. In repeated experiments, type A absorbed more of the lysin for sheep blood than did type B, which left it intact. This result, together with the other experiments, suggests a relation between the receptors of human blood, type A, and sheep blood. In serum 7, produced by immunization with type AB, the blood types A and B absorbed an equal amount of lysin for sheep blood, while type O absorbed much less. Absorption with sheep blood did not remove the lysin for human blood, but it removed completely the homologous antibodies. Previous investigators [8] also observed the inability of sheep blood to absorb lysins for human blood, type A, from heterophile immune serums. This is a point against the identity of the heterophile antigens in human blood A and in sheep blood.

The toxicity for guinea-pigs is considered an important quality of the heterophile immune serums. The lowest fatal dose reported in the literature was 0.03 cc. per hundred grams of the guinea-pig. However, it was observed, repeatedly, that a quantitative relation between the titer of the lysins for sheep blood and the toxicity for guinea-pigs does not exist; powerful heterophile immune serums were obtained that did not have any toxicity for guinea-pigs. Witebsky [7] produced a heterophile immune serum with human blood A which was toxic for guinea-pigs. The other investigators who worked with human blood did not mention whether or not they tested their immune serums for toxicity.

Fifteen guinea-pigs were injected intravenously with the various immune serums and with normal rabbit serum. The serums were inactivated for one-half hour at 55 C. and preserved with 0.5 cc. of a 5 per cent solution of phenol per ten cubic centimeters. Three rabbits received 2 cc. per hundred grams of body weight, the others 1 cc. In my experiments, there was hardly any difference between the toxic effect of the heterophile immune serum 1, that of the other immune serums, and that of normal rabbit serum. All guinea-pigs showed reactions of various degrees, but all survived. I did not find any difference in toxicity between fresh and inactivated normal rabbit serum.

Fujita,[8] who discovered the presence of heterophile antigen in Shiga's dysentery bacilli also noted the weak toxicity of his immune serum for guinea-pigs. The discrepancy can be explained by the assumption of various partial antigens, of which the human blood, type A, contains a much smaller number than do the organs of guinea-pigs.

The immune serums were titrated with all four types of human blood to determine whether they showed specificity for the group. Only serum 1 had a somewhat higher titer for the homologous blood, type A, the specificity for the group being more pronounced for the agglutinins than for the hemolysins. The results have been the same on repeated titrations. The other immune serums did not show any difference in relation to the various blood groups. Whether serum 1 can be considered group-specific is debatable, especially in view of the fact that absorptions with human blood, type B, removed hemolysins both for types A and B, and so did absorptions with type A. Kolmer and Trist[9] reported similar experiences in 1920.

The immune serums were tested repeatedly for heterophile precipitation with alcoholic extracts of the kidneys of guinea-pigs and rabbits. The alcoholic extract was diluted 1:6 with sodium chloride solution; 0.25 cc. of it was blown with a pipet into the immune serums, which were used in decreasing quantities from 0.2 to 0.01 cc. Precipitation was not observed with either the alcoholic extract of the guinea-pig kidney or of the rabbit kidney.

None of the immune serums hemolyzed or agglutinated ox blood. Isophile sheep hemolysins, which were tested at the same time, showed a marked degree of hemolysis for ox blood.

## SUMMARY

1. Two rabbits into which human blood, type A, had been injected, developed heterophile immune serum with a relatively high lytic titer for sheep blood. Six rabbits immunized with human blood, types O, B

8. Fujita, K.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **41**:539, 1924; **43**:329, 1925.

9. Kolmer, John A., and Trist, Mary E.: J. Immunol. **5**:89, 1920.

and AB, did not show any development of lysin for sheep blood; their natural hemolysin for sheep blood had qualities similar to the immune heterophile hemolysin for sheep blood.

2. The heterophile immune serum did not agglutinate sheep blood and did not hemolyze or agglutinate ox blood; it was slightly toxic for guinea-pigs, but not more so than normal rabbit serum. A precipitation was not observed when the serum was mixed with alcoholic extracts of the kidneys of guinea-pigs.

3. The hemolysin for sheep blood was removed by absorption with livers and kidneys of guinea-pigs, but not by absorption with livers and kidneys of rabbits. Absorption with human blood A removed some of the lysin for sheep blood; absorption with sheep blood did not influence the lysin for human blood A.

4. One of the serums obtained by immunization with type A hemolyzed and agglutinated blood A in a higher dilution than it did the other blood types.

## CONCLUSION

The results confirm the opinion of previous writers that the heterophile antigen present in human blood, type A, has common receptors with the Forssman antigen in sheep blood, but is not identical with it.

# Laboratory Methods and Technical Notes

## A GRAPHITE PREPARATION FOR INTRAVITAL STAINING*

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In a recent publication (1927), Drinker and Churchill [1] described a method for the preparation of a suspension of finely particulate graphite, commercially known as Hydrokollag 300, which they employed, as a medium, for certain experiments in perfusion of the capillaries of a frog's foot. This colloidal preparation, composed of particles vastly smaller than blood cells, may be introduced into the blood stream for a given period and then washed completely out, as it does not adhere to the vascular walls. The preparation is therefore admirable for such experiments.

These investigators encountered difficulty, however, when they attempted to employ this suspension as an intravital stain. They found that if the preparation was injected intravenously into a frog, the particles of graphite began within a few minutes to be agglutinated into small clumps, which acted as emboli and caused death. The capillaries of the tongue of a frog were exposed, and the suspension of graphite was injected directly into the femoral vein. Immediately the capillaries became black, and the particles of graphite could be seen moving along with the corpuscles in the capillaries, without agglutination or visible blockage. However, within ten minutes these small particles began to collect in irregular masses, which gradually increased in size and formed emboli. Such agglutination continued in the frogs, even when heparin had been given intravenously, although it did not occur when the graphite was mixed with blood containing heparin and allowed to stand over night. Splenectomy or hepatectomy did not inhibit the agglutinative reaction. On the basis of these observations, Drinker and Churchill concluded that this suspension of particulate graphite is not suitable for intravital staining of the phagocytic cells of the animal body.

In view of the extensive staining reactions which follow intravenous injection of many of the more common vital dyes, it is highly desirable that some inert finely particulate material, such as this graphite, be available for intravital use. Accordingly, we outline, briefly, a simple method by which this suspension of graphite may be used as an intravital stain, and its intravenous administration continued in increasing doses

* From the Division of Experimental Surgery and Pathology, The Mayo Foundation.

1. Drinker, C. K., and Churchill, E. D.: Graphic Suspension for Intravital Injection of Capillaries, Proc. Roy. Soc. Med. **101**:462, 1927.

over an indefinite period without apparent agglutination or formation of emboli. The final preparation used in our observations on the reticulo-endothelial system is one prepared from the commercial product in a manner slightly different from that of Drinker and Churchill. The modification of their method, which we are using, has been described in detail in another report.[2]

From previous observations, it appeared to us that a satisfactory method of staining could be developed, in which daily increasing intravenous administrations of the final preparation would inhibit the agglutinative reaction which normally follows a single large dose. Accordingly, ten rabbits were selected for this study. Half a cubic

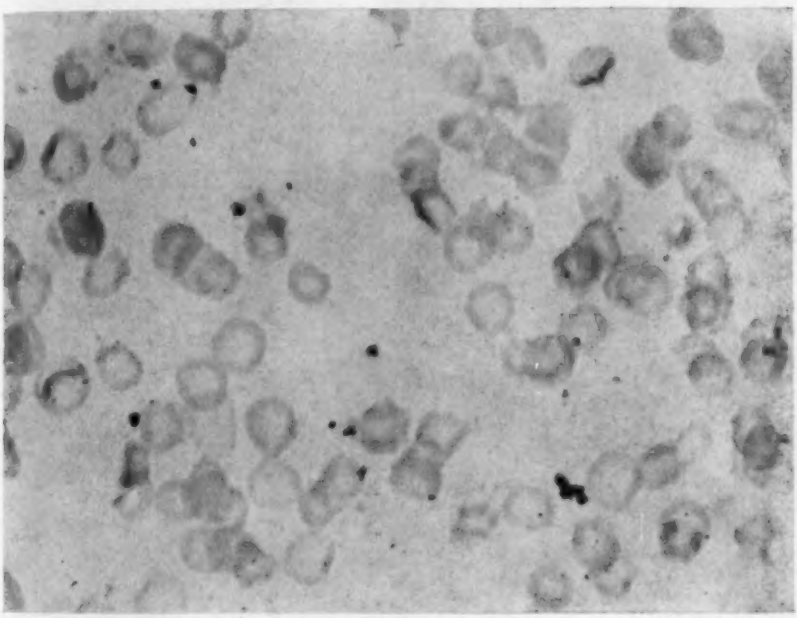

Fig. 1.—The peripheral blood of a rabbit with particles of graphite five minutes after the last of eight daily injections of Hydrokollog 300 (0.5 cc. on each of the first four days, then 1 cc. for each of two days, and 2 cc. on each of the last two days); × 800.

centimeter of the centrifugalized preparation was injected slowly into the marginal vein of the ear of each rabbit on four consecutive days. Smears of the blood made immediately after the injection showed that the blood was free of graphite within a few minutes. On the fifth day, the amounts injected were increased to 1 cc. without any apparent deleterious effect on the animal, and on the seventh day, the amount was increased to 2 cc. In certain animals of the series the daily

2. Higgins, G. M., and Murphy, G. T.: The Phagocytic Cells (v. Kupffer) in the Liver of Common Laboratory Animals, Anat. Record, to be published.

injections were increased to 3 cc., and still the animals did not appear to suffer ill effects.

After the fourth injection, an animal was killed daily and observations were made on the various organs of the body to determine the site of the pigment. Studies were also made on the content of the pigment in the blood following the daily injections. After the eighth or ninth injection, the blood stream did not clear as readily as it had earlier, and, even an hour after an injection, many discrete, extracellular particles of graphite were found free in the blood stream.

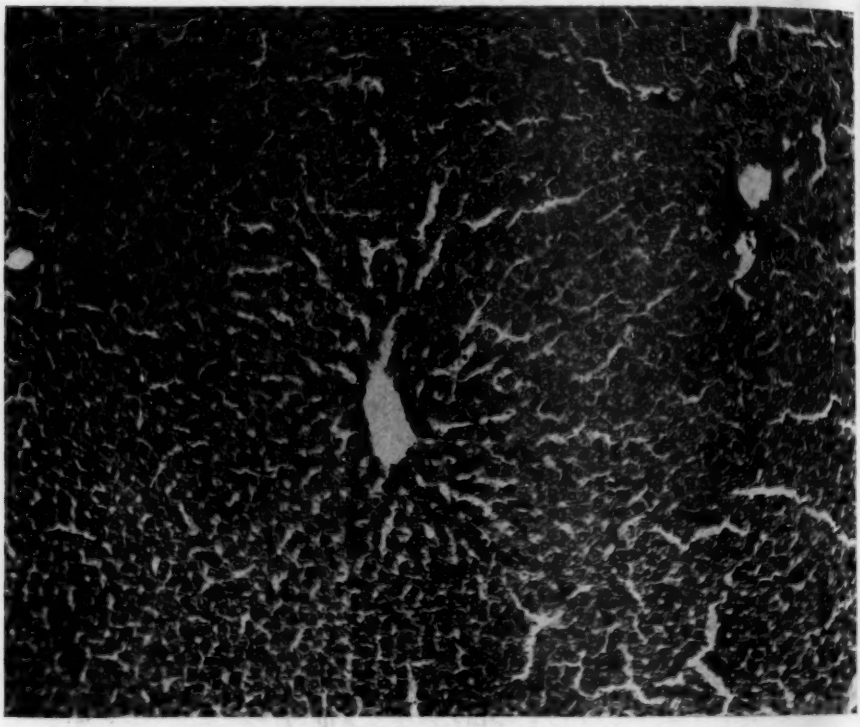

Fig. 2.—The liver of a rabbit showing Kupffer cells engorged with graphite after 7.5 cc. of the graphite suspension had been injected in nine daily intravenous injections of graduated amounts; × 350.

Figure 1 shows the extent of the distribution of graphite throughout the peripheral blood five minutes after the injection of 2 cc. on the eighth day.

In an animal receiving a total of 8 cc. of the suspension of graphite intravenously, over a period of nine days, the specialized cells comprising the reticulo-endothelial system were literally packed with dye, while the remaining portions of the body were entirely free. In the liver, the stellate cells were engorged with the particles, to such an extent that they frequently were found to exceed the hepatic cells in

Fig. 3.—The spleen of a rabbit after a total injection of 7.5 cc. of the graphite suspension, on the ninth day of daily administration of the preparation in graduated amounts; × 350.

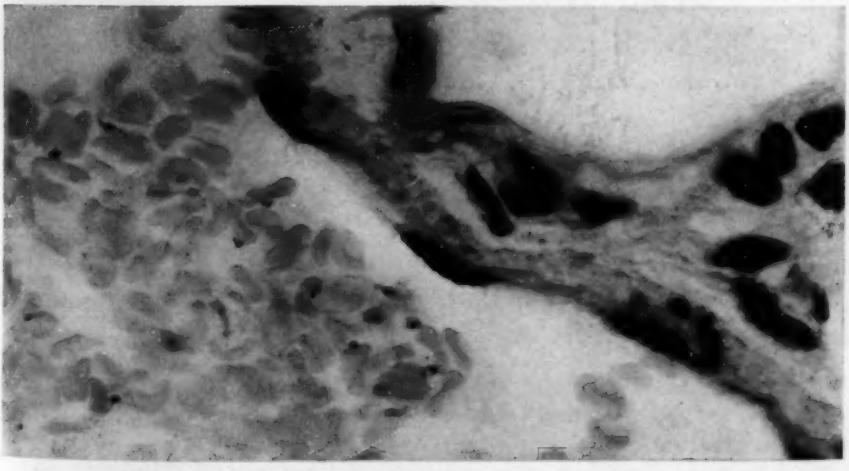

Fig. 4.—A blood vessel in a rabbit's lung, showing graphite present in the blood stream but absent from the endothelial cells; × 400.

size (fig. 2). In the spleen (fig. 3), the same reaction had taken place: the cells lining the splenic sinuses were packed with graphite. On the other hand, the regions of the body not normally possessing phagocytic functions did not contain particles of graphite even after many injections. Sections of the heart muscle and sections of the subcutaneous tissue were devoid of the injected material. Sections of the lung showed graphite in the larger blood vessels; but the endothelial cells (fig. 4) lining the vascular channels were devoid of the particles. The alveoli of the lung, however, showed these particles

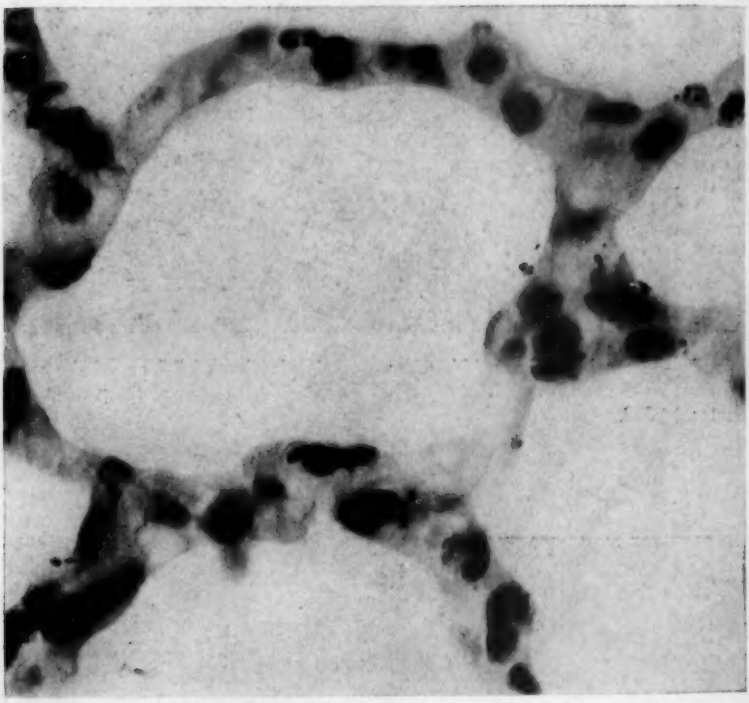

Fig. 5.—Alveoli of the same lung as shown in figure 4, showing particles of graphite present in the cells in the parenchyma; × 800.

distributed generally in cells throughout the parenchyma (fig. 5). Sections of the lung showed the occasional blood monocyte well distended with the preparation.

On the basis of these preliminary observations, therefore, we concluded that this particulate graphite, Hydrokollag 300, when properly administered, forms an entirely adequate medium for the intravital staining of phagocytic cells of the organism.

## THE NEW BUILDING FOR POSTMORTEM EXAMINATIONS AT DENVER

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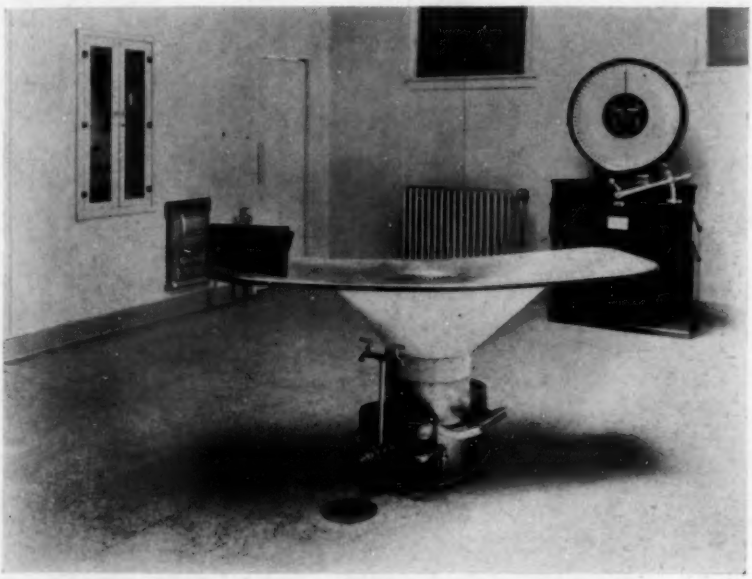
A new building for postmortem examinations has been furnished recently in Denver. It is on the grounds of the Denver General Hospital and serves the needs of the coroner and the hospital. To a receiving room, bodies are brought by the police in ambulances and put horizontally into a refrigerator, which will hold twenty bodies. From the hospital, the bodies come through a tunnel into the basement of the new building, and then by elevator into the receiving room.



The room for postmortem examinations in a new building especially designed for the purpose on the grounds of the General Hospital, Denver.

For each body there are separate doors and compartments in the refrigerator. The compartments open on the other side into the room for postmortem examinations. There is no connection between the receiving room and that for postmortem examinations, except through the office.

The necropsy table has a single central support on which it turns. It has a copper top and a central drain. Both hot and cold water are led into mixing chambers at the base of the table, and from there, with the temperature adjusted, the water is conducted to a cup on the foot of the table into and from which it runs constantly during examinations. By means of a foot lever, the table rests either on the floor or on one arm of a scale, which registers weights from one-eighth to 325 pounds. The weight register is at one side of the room close

to the wall; its entire arrangement is somewhat like that of the scales for weighing hay. As the table has measurements for length stamped in the copper top, it is thus possible to both weigh and measure bodies on the table. The windows are high, and over the table there is a skylight. The interior finishing, the plumbing, and the other appurtenances are all like those in modern surgical operating rooms.

The building has, also, a room fitted out with a table for embalmers; it has an incinerator, a rest room for visitors, an office, a large vault, several toilets and, in one lavatory, for the doctors, a shower bath. The roof of the building is well insulated.

# General Review

## MALARIA *

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From the earliest days of medical history, probably no subject has attracted more attention from the medical profession or the layman than the malarial fevers, because of their great economic importance and the fascinating problems connected with their etiology, epidemiology, prophylaxis and treatment. The discovery of the method of transmission of these infections by certain mosquitoes opened up the entire subject of the transmission of the diseases of man by insects, and was the starting point of research in this direction. The recent treatment in cases of paresis by the induction of malaria in the paretic patient through the inoculation of blood containing the malaria plasmodia, or the experimental production of the infection through the bites of infective mosquitoes, has resulted in the acquisition of a large amount of data relating to the etiology and epidemiology of malaria, and the discovery of new synthetic drugs, in the search for a substitute for quinine, has increased the resources of the physician in the prophylaxis and treatment of these important infections. In the following review of malaria, an endeavor has been made to record the most important advances that have been made in the study of the subject during recent years.

### ETIOLOGY AND EPIDEMIOLOGY

Much has been added to the knowledge of the etiology of the malarial fevers within the past decade, and there is a far clearer understanding than ever before of the factors controlling the production of the symptoms of infection and the transmission of the plasmodia. While this is true, however, recent work on these subjects has shown that only the threshhold has been reached of what must be acknowledged one of the most intricate and interesting problems in medicine, and the more the subject is studied the more one must realize how little is known of the numerous factors pertaining to the transmission of these important infections. While it is known that the plasmodia are transmitted from man to man through the agency of certain mosquitoes belonging to *Anophelinae*, the factors controlling such transmission are still only

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partly known, and it is probable that the knowledge at hand is fragmentary and unimportant when compared with the many factors which are still unknown.

*Classification of the Plasmodia.*—Although the malaria plasmodia were discovered by Laveran, in 1880, there is still some confusion in their zoological classification, and protozoologists still differ in their opinions regarding the exact zoological position of the parasites. In 1885, Marchiafava and Celli [1] placed these organisms in a new genus which they called *Plasmodium,* which included all of the differentiated species then known. In 1889, Grassi and Feletti [2] divided the genus *Plasmodium* into two genera, *Haemamoeba* and *Laverania.* In the genus *Haemamoeba* they included all malaria plasmodia of spherical shape, and in the genus *Laverania* all malaria plasmodia of crescentic shape. They believed the "crescents" or gametocytes of the estivo-autumnal plasmodium, *Plasmodium falciparum,* to be a distinct genus, when, as a matter of fact, these forms were only one stage in the life cycle of the parasite. It will thus be seen that this genus was improperly established, as it included only one stage in the development of the well known *Plasmodium falciparum.* Some recent writers, as Thomson and Woodcock [3] and Manson-Bahr,[4] accept the genus *Laverania* and include in it *Plasmodium falciparum* under the name *Laverania falcipara,* but most authorities do not recognize this genus and prefer to consider that all of the known malaria plasmodia of man belong in the genus *Plasmodium.* It is considered doubtful if a difference in the shape of the gametocytes is sufficient on which to base a new genus, and as the name *Plasmodium* has become firmly fixed in the nomenclature, and as only confusion will result by adopting the generic name *Laverania,* it is believed that the latter name should be abandoned and all of the species of malaria plasmodia retained in the genus *Plasmodium.* This opinion is concurred in by Wenyon [5] (1926), who, in his classical work on protozoology, says:

> As Schaudinn [6] pointed out, the mere difference in the shape of a gametocyte is not in itself sufficient to justify a separate generic name. The correspondence in the life-cycle is so close that it seems clear that the parasite of malignant tertian malaria must be included in the same genus as the other malarial parasites of man.

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1. Marchiafava and Celli: Fortschr. d. Med. **24**:787, 1885.
2. Grassi and Feletti: Arch. ital. de biol. **13**:297, 1890; Centralbl. f. Bakteriol. **7**:396, 430, 1890.
3. Thomson and Woodcock in Byam and Archibald: The Practice of Medicine in the Tropics, New York, Oxford University Press, 1922, vol. 2, p. 1516.
4. Manson-Bahr, P. H.: Manson's Tropical Diseases, ed. 8, New York, William Wood & Company, 1925, p. 27.
5. Wenyon, C. M.: Protozoology, New York, William Wood & Company, 1926, vol. 2, p. 934.
6. Schaudinn, F.: Arb. K. Gsndhtsamte. **19**:169, 1902.

*Unity or Plurality of Species of Malaria Plasmodia.*—The question of the unity or plurality of species among the plasmodia of malaria has always been a controversial question, and it will be remembered that Laveran, the discoverer of these parasites, always believed that there is only one species of plasmodium and that the various morphologic forms observed in the blood were simply variations brought about by environmental factors which are not known. The belief in the existence of only one species of plasmodium received an impetus during the World War, when several observers described what they regarded to be transmutations of the three species generally accepted by protozoologists and students of malaria. This evidence rested on the finding of one species in the blood after an attack caused by another of the accepted species, but double infections could not be excluded and in not a single instance could a true transmutation of species be scientifically demonstrated. At the present time practically every student of the subject believes that there are several species of malaria plasmodia, for the experimental evidence to this effect is incontrovertible and can be explained only by the existence of such species.

The species that are well differentiated and are accepted by practically all authorities are the following: *Plasmodium vivax,* the benign tertian plasmodium; *Plasmodium malariae,* the quartan plasmodium; and *Plasmodium falciparum,* the malignant tertian, or estivo-autumnal plasmodium.

The evidence on which the demonstration of distinct species of malaria plasmodia rests consists in the results obtained by the direct inoculation into man of blood containing each species and the experimental transmission of each species to man by the mosquito. Gerhardt [7] was the first successfully to produce malaria in man by the direct inoculation of blood containing the benign tertian plasmodium, and his observations were followed by those of Gualdi and Antolisei,[8] who produced quartan and estivo-autumnal infection in man by the inoculation of blood containing the quartan and estivo-autumnal plasmodia, respectively. These observations were soon confirmed by numerous investigators, and before 1910, according to Ross [9] (1911), there were recorded more than fifty experimental cases of malaria produced by the inoculation of blood containing the plasmodia. In every instance, the species of plasmodium inoculated appeared in the blood of the inoculated subject and that species only.

While the evidence at hand prior to 1922 was amply sufficient to prove the existence of at least three distinct species of malaria plas-

7. Gerhardt, P.: Ztschr. f. klin. Med., 1884, p. 375.

8. Gualdi and Antolisei: Riforma Med., no. 264, p. 1580; no. 274, p. 1639, 1889.

9. Ross, R.: The Prevention of Malaria, ed. 2, New York, E. P. Dutton & Company, 1911.

modia, it has been placed on an unassailable foundation through the recent treatment of paretic patients by malaria, based on the inoculation of the patient with blood containing the various malaria plasmodia, or the experimental infection of paretic persons by the bites of mosquitoes infected with the various species of plasmodia. Doerr and Kirschner [10] passed the same strain of the tertian plasmodium (*Plasmodium vivax*) through sixty-two persons during thirteen consecutive months, 240 generations of the plasmodium occurring during that time. There was not the slightest change in morphology during the entire time, and the plasmodium bred true in every generation. Wagner-Jauregg [11] transmitted the tertian plasmodium to over 200 persons, and in no instance did any plasmodia differing from *P. vivax* in morphology or life cycle appear in the blood of the inoculated persons. Mühlens and Kirschbaum,[12] during fifteen consecutive months, transmitted the same strain of the tertian plasmodium to fifty-nine persons; *P. malariae*, the quartan plasmodium, to five; and *P. falciparum*, the tertian estivo-autumnal plasmodium, to nine, and in no case was there any evidence of mutation of species, each plasmodium breeding true morphologically and clinically. Grant and Silverston,[13] for nearly four years, used the same strain of the tertian plasmodium in the treatment of patients with paresis, and during that time 60 direct transmissions, covering 150 individuals, were made with the organism. They state that there was no change in the morphology of the parasite. During this time over 10,000 blood films, taken at all stages of the development of the parasite, were examined, and changes from the typical morphology of *Plasmodium vivax* were not noted in any.

The evidence as to the plurality of species furnished by the direct transmission of the various species of plasmodium from man to man by inoculation of infected blood is confirmed by the observations on experimental malaria produced through the bites of mosquitoes that have bitten persons infected with the three species of malaria plasmodia mentioned. Thus, Yorke and Macfie,[14] James,[15] St. John [16] and many others have shown that the morphology of the particular species experimented with never changes, no matter how many times it is passed through the mosquito and the human host, so that today no well informed person doubts the existence of the three species of malaria

10. Doerr and Kirschner: Ztschr. f. Hyg. u. Infectionskrankh. **92**:279, 1922.
11. Wagner-Jauregg, J.: J. Nerv. & Ment. Dis. **55**:369, 1922.
12. Mühlens and Kirschbaum: Arch. f. Schiffs- u. Tropen- Hyg. **28**:131, 1923.
13. Grant and Silverston: J. Trop. Med. **29**:117, 1926.
14. Yorke and Macfie: Tr. Roy. Soc. Trop. Med. & Hyg. **18**:13, 1924.
15. James, S. P.: Tr. Roy. Soc. Trop. Med. & Hyg. **20**:143, 1926.
16. St. John, J. H.: Am. J. Trop. Med., to be published.

plasmodia which have been mentioned, i.e., *Plasmodium vivax, Plasmodium malariae* and *Plasmodium falciparum.*

*Other Species of Malaria Plasmodia.*—In recent years, a great deal of evidence has been accumulated which tends to prove that there are other species of malaria plasmodia existent which are not generally recognized, and several such species have been described by different observers. Aside from the morphologic evidence brought forward in favor of such a view, it is evident that the clinical symptoms of malarial infection in certain regions lend support to this conception. It is well known, for instance, that in certain regions malarial infections, presumably caused by *Plasmodium falciparum,* are much more fatal than in other regions in which the climate is similar and in which the local conditions are practically identical. That these fatal estivo-autumnal infections may be due to a different variety, or species, of plasmodium, is not beyond reason, and the fact that the plasmodia present in persons in such regions differ in morphology from the typical *Plasmodium falciparum* would appear to be sufficient evidence on which to base the description of a new variety or species. Grassi and Feletti,[17] Mannaberg[18] and Manson[19] all recognized more than one species of the estivo-autumnal plasmodium, but the clearest description of the different species of this type of plasmodium was given by Marchiafava and Bignami,[20] who recognized two species of estivo-autumnal plasmodia, a quotidian and tertian species.

In 1901, I[21] confirmed the observations of Marchiafava and Bignami, and described two species of plasmodia associated with the estivo-autumnal infections that I studied and that originated in Cuba and the Philippine Islands, one segmenting in twenty-four hours and the other in forty-eight hours, each causing a febrile paroxysm at the time of sporulation. In 1909, I proposed the name *Plasmodium falciparum quotidianum* for the parasite segmenting every twenty-four hours and

17. Grassi and Feletti (footnote 2, second reference).

18. Mannaberg, J.: Die Malaria Parasiten, Vienna, Alfred Hölder, 1893.

19. Manson, P.: Tropical Diseases, New York, William Wood & Company, 1907.

20. Marchiafava and Bignami: Deutsche med. Wchnschr. **18**:1157, 1188, 1892; Malaria, Twentieth Century Practice, New York, William Wood & Company, 1900, vol. 19.

21. Craig, C. F.: The Aestivo-autumnal (Remittent) Malarial Fevers, New York, William Wood & Company, 1901; Boston M. & S. J. **160**:677, 1909; The Malaria Fevers, Haemoglobinuric Fever and the Blood Protozoa of Man, New York, William Wood & Company, 1909; Am. J. Trop. Med. **1**:57, 1921; The Malarial Fevers in Osler and McCrae: Modern Medicine, Philadelphia, Lea & Febiger, ed. 3, 1926; Malaria in Christian and Mackenzie: Oxford Medicine, New York, Oxford University Press, 1927; A Manual of the Parasitic Protozoa of Man, Philadelphia, J. B. Lippincott Company, 1926.

producing a quotidian fever, regarding it as a subspecies or variety of the tertian estivo-autumnal plasmodium, or *Plasmodium falciparum*. The two plasmodia differ in their morphology, the time consumed in their life cycle in man, and in the type of clinical malaria which they produce. Bass,[22] who was able to distinguish these two plasmodia in cultures, states "that there are at least two and probably more subdivisions of the estivo-autumnal parasite." While *Plasmodium falciparum quotidianum* is not, as yet, generally recognized, the evidence is sufficient to justify its being regarded as a subspecies, at least, of *Plasmodium falciparum*, and it is believed that more careful research and study of the morphology of the estivo-autumnal plasmodia will result in the general recognition of this subspecies.

In 1900, I[23] published a description of a plasmodium observed in the blood of infected American soldiers returning from the Philippines, which was regarded as either a distinct variety of the malaria plasmodia, or a tertian plasmodium in which the morphology had become changed in some unknown manner. This same plasmodium was again described by Ahmed Emin,[24] in 1914, as a variety of the tertian plasmodium, and was named by him *Plasmodium vivax* variety *minuta*. His description agrees with mine, and there is little doubt that this parasite is a distinct variety, or subspecies of *Plasmodium vivax*.

In 1922, Stephens[25] described a plasmodium occurring in the blood of a patient from East Africa, which apparently agrees in its morphology with *Plasmodium vivax* variety *minuta*, and which he has called *Plasmodium ovale*. It is probable that *Plasmodium ovale* is identical with *Plasmodium vivax* variety *minuta*, although Stephens believes that the two parasites are distinct; in a recent paper Stephens and Owen[26] state that they could not identify it with *Plasmodium vivax minutum*.

Another species of malaria plasmodium has been described by Stephens[27] in blood smears from a patient suffering from malaria in the Central Provinces, India. This species, which he has called *Plasmodium tenue*, resembles *Plasmodium falciparum*, but the young intracellular forms are ameboid, and in stained preparations differ markedly in morphology from similar stages in the development of *Plasmodium falciparum*. The observations of Stephens have recently been confirmed

22. Bass, C. C., Malaria, in Tice: Practice of Medicine, Hagerstown, Md., W. F. Prior Company, 1920, vol. 3.

23. Craig, C. F.: Rep. Surg.—Gen. Army, Govt. Printing Office, Washington, D. C., 1900, p. 59.

24. Emin, A.: Bull. Soc. path. Exot. **7**:385, 1914.

25. Stephens, J. W. W.: Ann. Trop. Med. **16**:383, 1922.

26. Stephens, J. W. W., and Owen: Ann. Trop. Med. **21**:293, 1927.

27. Stephens, J. W. W.: Proc. Roy. Soc. **87**:375, 1914; Ann. Trop. Med. **8**: 119, 1914.

by Sinton,[28] and there can be no doubt that the forms described by these investigators do occur in some estivo-autumnal infections, but many observers regard them as atypical forms of *Plasmodium falciparum*. Recently, Callanan[29] published a paper describing similar parasites which he believes were merely young ring-forms of *Plasmodium falciparum* in which the "ring" had been ruptured by pressure on the corpuscles during the preparation of the films, and he thus explains the occurrence of the tenue forms. The question as to the specific status of *Plasmodium tenue* is still undecided.

In 1915, Ziemann[30] described a new species of malaria plasmodium causing pernicious malaria in West Africa, which he called *Plasmodium perniciosum*. This so-called species has been studied by Wenyon,[31] who states that the differential features claimed by Ziemann are not always present, and that he believes "there is little reason for regarding *Plasmodium perniciosum* as a distinct species."

It is the part of wisdom to inquire carefully into the validity of new species of malaria plasmodia before denying their existence, for there is every reason to believe that the possibilities in this respect have not been exhausted. It is interesting to recall the recent discovery that *Plasmodium praecox*, causing malaria in birds, really included, under this name, three different species of plasmodia, for Hartman[32] has demonstrated that three distinct species of plasmodia are concerned in the production of malaria in birds, and that all three have been confused in the past under the name, *Plasmodium praecox*.

The suggestion of Marchoux[33] that there exists different races of the three well known species of malaria plasmodia which vary in their geographical distribution, morphology and in the clinical symptoms which they produce deserves consideration in view of what is known regarding the occurrence of different races among the bacteria and some of the protozoa.

*Malaria Plasmodia in Lower Animals.*—The possible occurrence of the malaria plasmodia of man in the lower animals has always been a subject of much research. The occurrence of malarial fevers in so-called uninhabited regions, or in regions in which there are few human inhabitants, has been explained by some writers as due to the presence in such regions of some animal host of the plasmodia, and the search for such a host has been greatly stimulated by the discovery that plasmodia do

28. Sinton, J. A.: Indian. J. M. Research **10**:203, 215, 1922.
29. Callanan, J. C. J.: Kenya Med. J. **3**:182, 1926.
30. Ziemann, H.: Centralbl. f. Bakteriol. **76**:385, 1915.
31. Wenyon, C. M.: Human Protozoology, New York, William Wood & Company, 1926, vol. 2.
32. Hartman, E.: Arch. f. Protistenk. **60**:1, 1927.
33. Marchoux, E.: Proc. First International Malaria Congress, Rome, 1925.

occur in several of the lower animals that are morphologically indistinguishable from the species occurring in man.

Numerous species of plasmodia occur in the blood of birds, but those occurring in mammals, especially in monkeys and the chimpanzee and orang-utan, are of special interest to the malariologist.

In 1899, Dionisi [34] described two plasmodia occurring in the blood of bats which he named *Plasmodium murinum* and *Plasmodium melanipherum*, resembling the quartan plasmodium of man, *Plasmodium malariae*, in their morphology. A similar plasmodium, *Plasmodium brodeni*, was discovered, in 1913, in the blood of the jumping rat of the Belgian Congo by Rodhain, Baquaert, Pons and Vandenbranden,[35] while in 1915, Rodhain [36] described a similar parasite in the blood of the flying fox in the same locality. This animal is also parasitized by another plasmodium resembling *Plasmodium vivax* of man, which Breinl [37] discovered in 1912, and named *Plasmodium pteropi*. Laveran studied a plasmodium discovered by Vassal [38] in the squirrel of Annam, which he named *Plasmodium vassali*. This plasmodium resembled *Plasmodium malariae* of man in morphology, but it was not inoculable into man or into other animals. A similar plasmodium was found in the blood of the squirrel in India by Donovan,[39] which he named *Plasmodium ratufae*.

Several different species of plasmodia have been described in larger mammals, exclusive of monkeys and apes. Bruce and his co-workers [40] found a plasmodium in the blood of the duiker, a species of antelope in Africa, resembling *Plasmodium malariae* in morphology, which they named *Plasmodium cephalophi*. Sheather,[41] in India, found a plasmodium in the blood of a buffalo resembling *Plasmodium malariae* of man, which he called *Plasmodium bubalis*. De Mello and Paes [42] described a plasmodium in the blood corpuscles of goats, which they named *Plasmodium caprae* and which morphologically resembled *Plasmodium falciparum*, the estivo-autumnal plasmodium of man. Castellani and Chalmers [43] have described a plasmodium in dogs in India which they

34. Dionisi, A.: Arch. ital. de biol. **31**:153, 1899.

35. Rodhain; Baquaert, Pons and Vandenbranden: Bull. Soc. path. Exot. **6**: 182, 1913.

36. Rodhain, J.: Bull. Soc. path. Exot. **8**:726, 1915.

37. Breinl, A.: Rep. Australian Inst. Trop. Med., 1912.

38. Vassal, J. J.: C. R. Soc. Biol. **58**:350, 1905.

39. Donovan, C.: Indian J. M. Research **7**:717, 1920.

40. Bruce, D.; Harvey, Hamerton and Lady Bruce: Proc. Roy. Soc. **87**:45, 1913.

41. Sheather, A. L.: J. Comp. Path. & Therap. **32**:223, 1919.

42. De Mello and Paes: C. R. Soc. Biol. **88**:829, 1923.

43. Castellani and Chalmers: Manual of Tropical Medicine, ed. 1, New York, William Wood & Company, 1910.

named *Plasmodium canis,* resembling *Plasmodium vivax* of man in morphology, and another species in the horse which they named *Plasmodium equi.*[44] Neither of these species has been confirmed by other observers.

The species of malaria plasmodia occurring in monkeys, chimpanzees and orang-utans have attracted much attention because it has been thought that perhaps these animals act as hosts or "carriers" of the plasmodia of man, and that the species found in these animals may be identical with those occurring in man. Koch [45] was the first to observe a malaria plasmodium in the monkey, and Laveran named the species *Plasmodium kochi.* This plasmodium has been repeatedly studied by different observers and is a common parasite of monkeys belonging to the genera *Cercopithecus, Cercocebus* and *Cynocephalus.* Its life cycle is completed in forty-eight hours, and in morphology it is practically identical with *Plasmodium vivax* of man. It is only slightly pathogenic to its hosts and can be destroyed by quinine. Attempts to infect man with this species have been unsuccessful. Two other species of plasmodium have been described in monkeys of the genus *Macacus* which closely resemble *Plasmodium vivax* of man in morphology. These are *Plasmodium inui,* described by Halberstädter and Prowazek,[46] in 1907, and *Plasmodium cynomolgi,* described by Mayer,[47] in 1908. It is practically certain that the latter species is identical with *Plasmodium inui.* A similar plasmodium has been described by Knowles [48] under the name *Plasmodium semnopitheci,* in the blood of a monkey belonging to the genus *Semnopithecus.*

A species resembling in its morphology *Plasmodium malariae,* the quartan malaria plasmodium of man, has been found in the blood of a monkey belonging to the genus *Brachyurus,* from the Amazon region, by Gonder and Gossler.[49] This plasmodium, which they named *Plasmodium brasilianum,* is almost identical in morphology with *Plasmodium malariae.*

Species of plasmodia resembling the three species causing malaria in man have been found in the blood of anthropoid apes, as chimpanzees, orang-utans and the gorilla, and have been studied especially by Reichenow and by Blacklock and Adler. In 1917, Reichenow [50] published a description of plasmodia which he found in chimpanzees in the

44. Castellani and Chalmers: Manual of Tropical Medicine, ed. 2, New York, William Wood & Company, 1913.

45. Koch, R.: Centralbl. f. Bakteriol. **24**:200, 1898.

46. Halberstädter and Prowazek: Arb. K. Gsndhtsamte. **26**:37, 1907.

47. Mayer, M.: Arch. f. Protistenk. **12**:314, 1908.

48. Knowles, R.: Indian J. M. Research **7**:195, 1919.

49. Gonder and Gossler: Malaria, **1**:47, 1908.

50. Reichenow, E.: Bol. R. Soc. Espan. Hist. Nat. **17**:312, 1917.

Cameroons, but his observations in this respect were antedated by those of Halberstädter and Prowazek who found plasmodia in the blood of the orang-utang and the chimpanzee resembling both *Plasmodium vivax* and *Plasmodium malariae* of man. They gave the name, *Plasmodium pitheci*, to this plasmodium. In 1920, Reichenow [51] stated that of eight champanzees examined by him, five showed plasmodia in their blood that were indistinguishable morphologically from *Plasmodium falciparum*, the estivo-autumnal plasmodium of man, while one showed plasmodia indistinguishable from *Plasmodium vivax*, and two, plasmodia indistinguishable from *Plasmodium vivax, Plasmodium malariae* and *Plasmodium falciparum*. The plasmodium resembling *Plasmodium falciparum* was named *Plasmodium reichenowi* by Sluiter, Swellengrebel and Ihle,[52] although Reichenow believed it to be identical with the plasmodium occurring in man.

Further observations on the plasmodia of chimpanzees have been contributed by Blacklock and Adler,[53] who found plasmodia resembling *Plasmodium malariae* in morphology, but forms also occurred resembling *Plasmodium falciparum* and *Plasmodium vivax*. They were unable to infect two human beings by inoculations of blood subcutaneously and intravenously with these plasmodia, and later,[54] were unable to infect a chimpanzee by the inoculation of blood from man containing *Plasmodium falciparum*. In 1923, Adler [55] examined thirteen chimpanzees in Sierra Leone, and found one infected with a plasmodium resembling *Plasmodium falciparum* in morphology.

The relation of the plasmodia which infect the anthropoid apes to those causing malaria in man is still an undecided problem. Reichenow believes that the plasmodia in the chimpanzees are identical with those in man, and he regards these apes as possibly important sources of infection for man. These animals often live in close contact with human habitations and are apt to be bitten by infected mosquitoes, and, if the human plasmodia can live in their blood, there is no reason to doubt that these animals might be "carriers" of the parasites. However, all attempts to infect man with the plasmodia present in the blood of chimpanzees have resulted in failure and in only one instance was the attempt to infect the chimpanzee with a plasmodium of man successful, that of Mesnil and Roubaud,[56] who claim to have been successful in inoculating a chimpanzee with *Plasmodium vivax*, the tertian plasmodium of

51. Reichenow, E.: Centralbl. f. Bakteriol. **85**:207, 1920.

52. Sluiter; Swellengrebel and Ihle: De dierlijke parasiten van den mensch en van onze huisdieren, ed. 3, Amsterdam, 1922, p. 121.

53. Blacklock and Adler: Ann. Trop. Med. **16**:99, 1922.

54. Blacklock and Adler: Ann. Trop. Med. **18**:1, 1924.

55. Adler, S.: Ann Trop. Med. **17**:13, 1923.

56. Mesnil and Roubaud: Ann. de l'Inst. Pasteur **34**:466, 1920.

man. It must be admitted that plasmodia occur in the red blood corpuscles of chimpanzees, the orang-utan and the gorilla, which are indistinguishable, so far as morphology is concerned, from the three common species of malaria plasmodia occurring in man, but it is still undetermined whether these plasmodia are really identical with the plasmodia of man. In the chimpanzees, the pathologic picture in the viscera is identical with that encountered in infection in man, the organs being pigmented, while the plasmodia occur in large numbers in the spleen, liver and bone marrow. Continued research will undoubtedly solve this important problem, but it cannot be stated, at present, that any of the plasmodia found in any of the lower animals, are identical with those that cause malaria in man.

*Morphology of Plasmodia in Man and Mosquito.*—No definite contributions have been made during recent years, to the knowledge of the morphology of the malaria plasmodia, either in man or in the mosquito, with the exception of the description of certain forms by Grassi [57] and Schaudinn [58] as parthenogenetic forms of the parasites, and certain forms described by Craig [59] and by James [60] as possibly "latent forms" concerned in the production of relapse.

In 1900, Grassi noted changes in the female gametocyte of *Plasmodium vivax* which he interpreted as parthenogenetic and, in 1902, Schaudinn described similar changes which he believed indicated that, under certain conditions, the macrogametocyte, or female gametocyte, underwent segmentation into daughter individuals, and that these infected the red blood corpuscles, thus bringing about a relapse of the malarial symptoms. This theory has been accepted by numerous investigators, especially in France and Germany, although it is against all known biologic law, so far as the malaria plasmodia are concerned, and has been proved erroneous by Wenyon,[61] Thomson [62] and others. The so-called parthenogenetic gametocytes described by Schaudinn were probably instances of infection of a single red blood corpuscle with a segmenting schizont and a gametocyte.

It has been shown repeatedy that the injection of blood containing only gametocytes into man is not followed by infection and the occurrence of symptoms of malaria, so that there is no experimental evidence available that parthenogenesis occurs or is the cause of relapse in malarial disease.

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57. Grassi, B.: Die Malaria, Jena, 1900.
58. Schaudinn, F.: Arb. K. Gsdhtsamte. **19**:169, 1902.
59. Craig, C. F.: Philippine J. Sc. **1**:523, 1907; J. Infect. Dis. **4**:108, 1908.
60. James, S. P.: J. Roy. Army Med. Corps. **29**:317, 1917.
61. Wenyon, C. M.: Protozoology, 1926, vol. 2, p. 922.
62. Thomson, J. D.: J. Roy. Army Med. Corps **29**:379, 1917.

In 1906, I [59] described certain forms of the malaria plasmodia occurring in the blood of patients suffering from relapses which were apparently produced by the conjugation of the young schizonts, or ring-forms, and which I regard as "latent or resting forms" of the plasmodia which, under certain conditions, segmented and produced a relapse. The forms described differed in morphology from the forms of the plasmodia occurring in the human life cycle of the plasmodia as usually described and, when fully developed, somewhat resembled the gametocytes of the various plasmodia. A full description of the morphology of these forms has been published elsewhere.[59] S. P. James [60] has described the same forms, apparently, and considers that they are concerned in the production of relapse.

In a recent paper, St. John [16] has invesitgated the staining reactions of the gametocytes of *Plasmodium vivax* and has called attention to the fact that the usual description of these reactions is frequently incorrect, especially as regards the staining reactions of the male gametocyte, or microgametocyte. In most descriptions it is stated that the cytoplasm of this form of *Plasmodium vivax* stains a pale blue or a greenish blue, with the various modifications of the Romanowsky stain, as the Wright stain, but St. John has shown that the cytoplasm of the microgametocyte of *Plasmodium vivax* usually stains a marked purple or lilac, while that of the female gametocyte, or macrogametocyte, stains sky blue. While this is true in many instances, it is also true that the pale blue or greenish staining of the cytoplasm of the microgametocyte is frequently observed, and it is probable that the purple or lilac coloring occurs in the older microgametocytes in which the chromatin is large in amount and has become more generally distributed in the cytoplasm preparatory to flagellation, or the production of the microgametes. These differences in the staining reactions of the microgametocyte should be remembered in differentiating it from the macrogametocyte.

*Time of Occurrence of Gametocytes in the Peripheral Blood.*—The generally accepted statement that the gametocytes, or sexual forms, of the malaria plasmodia, which undergo their development in the mosquito, do not appear in the peripheral blood until from nine to twelve days have elapsed after the appearance of symptoms of the infection in primary cases, has been proved to be incorrect by observations made on the blood of patients suffering from paresis who have been experimentally infected by the bites of infected mosquitoes. That a malarial infection of sufficient intensity to cause symptoms is necessary before gametocytes are observed in the peripheral blood was shown to be an erroneous supposition by me,[63] when in 1910, I demonstrated that

63. Craig, C. F.: The Sexual Forms of the Malarial Plasmodia Occurring in the Blood of Man, Arch. Int. Med. **5**:325 (April) 1910.

gametocytes occurred in the peripheral blood of persons having a latent malarial infection, in whom no symptoms had ever occurred. This observation has been confirmed by many observers. While it is not true that symptoms must occur before gametocytes develop in man, it is true that in the vast majority of cases symptoms of malaria occur before gametocytes are observed in the peripheral blood. The date of appearance of the gametocytes after the development of symptoms is of much practical interest in the prophylaxis of malaria, and it has been definitely proved that these sexual forms of the plasmodia may occur soon after the appearance of clinical symptoms. Wenyon [64] states that they usually occur in from ten to fourteen days after the first attack of malaria, but that they have been observed at the time the first symptoms occurred. Yorke and Macfie,[65] in their experimental work in the treatment of patients with paresis by the induction of malaria by the bites of infected mosquitoes, found that gametocytes invariably appeared in the peripheral blood in from two to three days after the appearance of the shizonts, or human life cycle forms, and Stephens,[66] Thomson [67] and Lane [68] state that gametocytes may be found in the peripheral blood even before the schizonts are demonstrable. James,[15] in his observations on malaria produced experimentally by the bite of the infected mosquito, found that gametocytes never appeared in the peripheral blood before the seventh day after the first rise in temperature, and that the gametocytes were not infective to the mosquito until about three days later.

St. John [69] found that in malarial infections produced in paretic patients by the direct inoculation of blood containing the plasmodia, the gametocytes occurred early in the peripheral blood after the onset of the symptoms of the disease. In six cases, gametocytes occurred five days after the onset in one; three days after the onset in another; and two days after the onset in four. In a later contribution, St. John [16] states that gametocytes are present early in the course of tertian malaria initiated by mosquitoes, as he was successful in infecting mosquitoes from two different cases of malaria on the fourth day following the onset of the symptoms.

It is thus evident that the gametocytes may occur in malarial infections even before the occurrence of symptoms or within a day or two

64. Wenyon, C. M.: Protozoology, 1926.

65. Yorke and Macfie: Tr. Roy. Soc. Trop. Med. & Hyg. **18**:24, 1924.

66. Stephens, J. W. W.: Proc. Internat. Confer., Health Problems in Trop. America, 1924, p. 70.

67. Thomson, G.: Proc. Internat. Confer., Health Problems in Trop. America, 1924, p. 80.

68. Lane, C.: Trop. Dis. Bull. **33**:332, 1926.

69. St. John, J. H.: The Early Appearance of Gametocytes in Artificially Induced Tertian Malaria, J. A. M. A. **88**:1561 (May 14) 1927.

after such occurrence, so that one can never be sure that a patient showing symptoms of malaria is not infective to suitable mosquitoes at any time, unless a careful microscopic examination is made of the peripheral blood. This is a fact of great importance in prophylaxis, as will be mentioned later in this contribution.

*Cultivation of the Malaria Plasmodia.*—The first observer to cultivate successfully the malaria plasmodia was Bass,[70] who afterward, in conjunction with Johns,[71] perfected the technic and was able to cultivate all of the common species of the malaria plasmodia of man for from two to four or five generations, all stages in the human life cycle being observed in such cultures. For the technic of culturing these parasites, the reader is referred to work of Bass and Johns.[71] Their observations have been confirmed by numerous investigators and much has been added to this subject by Ziemann,[72] Thomson and Thomson,[73] Lavinder,[74] McLay [75] and Sinton.[28] All stages in the human life cycle of the various plasmodia may be seen in cultures, and in such cultures *Plasmodium vivax* segments in forty-eight hours, *Plasmodium malariae* in about seventy-two hours and *Plasmodium falciparum* in from twenty-four to forty-eight hours. The plasmodia segmenting in twenty-four hours were examples of *Plasmodium falciparum-quotidianum*, the quotidian estivo-autumnal plasmodium, in all probability.

Some observers have claimed to have found gametocytes in cultures, and Joukoff [76] and Perekropoff [77] state that the forms normally observed during the life cycle in the mosquito may also be observed in cultures, but their observations have not been confirmed and are considered erroneous. Sinton [78] and his co-workers describe the occurrence of large numbers of the crescentic gametocytes of *Plasmodium falciparum* and no asexual forms in a culture that had been kept in the incubator for ten days, although gametocytes had not been found in the patient's blood or in the culture during the seventy-two hours that it had been examined. This observation demonstrates that, under certain conditions, gametocytes may appear in cultures, but that such gametocytes will

70. Bass, C. C.: A New Conception of Immunity, J. A. M. A. **51**:1534 (Nov. 4) 1911.

71. Bass and Johns: J. Exper. Med. **16**:567, 1912.

72. Ziemann, H.: Arch. Schiffs- u. Tropen- Hyg. **17**:361, 1913.

73. Thomson and Thomson: Ann. Trop. Med. **7**:153, 509, 1913.

74. Lavinder, C. H.: A Note on the Cultivation of Malarial Plasmodia After the Method of Bass and Johns, J. A. M. A. **60**:42 (Jan. 4) 1913.

75. McLay, K.: J. Roy. Army Med. Corps **38**:93, 1922.

76. Joukoff, N. M.: C. R. Soc. Biol. **74**:136, 1913.

77. Perekropoff, G.: Arch. f. Protistenk. **25**:139, 1914.

78. Sinton, J. A.; Baily and Chandler: Indian J. M. Research **13**:895, 1926.

undergo further development in the cultures as they do in the mosquito is more than doubtful.

Owing to the difficulty of transferring the cultures and the fact that the plasmodia die out after a few generations, the cultivation of the malaria plasmodia has not been of much practical value in the study of malaria, but it is not too much to hope that more practical methods of cultivation will be developed, and if this can be accomplished one may look forward to an immense increase in the knowledge of the biology of these interesting and important parasites of man.

*Relation of the Malaria Plasmodia to the Red Blood Corpuscles.*—In 1912, Lawson,[79] from the study of stained blood smears containing malaria plasmodia, concluded that the parasites were extracellular in situation, although generally regarded as being intracellular. Again, in 1920,[80] she concluded that all stages of the plasmodia are merely attached to the red blood corpuscles, and that after the destruction of the cell by the parasite, it migrates to another cell and the process of destruction is repeated, so that one plasmodium may destroy several red blood corpuscles. Her observations were apparently confirmed by Sinton,[81] in 1922, who studied infected blood treated with hypertonic and hypotonic salt solutions and then sectioned the red blood corpuscles. They were also confirmed by DeLangen,[82] who washed infected red blood corpuscles with Ringer's solution and claimed to have greatly reduced the number of plasmodia per number of red blood corpuscles, and found that free plasmodia occurred in the washings. While these observations were interesting, to any one who had carefully studied the malaria plasmodia in fresh, unstained specimens of blood, the situation of the parasites was not in question, for in infections with *Plasmodium vivax*, one can often see the ameboid processes of the organism come up into view from the interior of the red blood corpuscle, thus demonstrating, beyond all doubt, the intracellular situation of the parasite. However, the recent observations of Ratcliffe [83] demonstate the intracellular situation of both *Plasmodium vivax* and *Plasmodium falciparum*, and there is no reason to believe that *Plasmodium malariae* or any other species of plasmodium differs from these two species as regards their location in the infected red blood corpuscle. By fixing fresh blood, sectioning

79. Lawson, M. Rowley: The Cause of Malarial Anemia and the Intravascular Migrations of the Malarial Parasite, Arch. Int. Med. **9**:420 (April) 1912.

80. Lawson, M. Rowley: J. Exper. Med. **31**:201, 1920.

81. Sinton, J. A.: Indian Med. Gaz. **57**:367, 1922.

82. DeLangen, C. D.: Tr. Sixth Cong. Far Eastern Assoc. Trop. Med. **2**:25, 1925.

83. Ratcliffe, H. L.: Am. J. Trop. Med. **7**:383, 1927; ibid., vol. 8, to be published.

it thin, and studying cross-sections of the red blood corpuscles, he has shown that the plasmodia were invariably intracellular in position.

*Method of Transmission.*—The only method of transmission of the malaria plasmodia from man to man, which has been scientifically demonstrated, is through the bite of mosquitoes that have fed on persons whose peripheral blood contains gametocytes of the malaria plasmodia. Such transference by the mosquito is only possible, so far as has been ascertained, after the gametocytes have undergone a cycle of development in the mosquito covering several days, resulting in the production of the infective forms, or sporozoites, which are present in large numbers in the salivary glands and ducts of the insect and which are injected into the blood of man when the insect bites. While the foregoing statement is presumably true, it can hardly be said that the possible direct mechanical transmission of the plasmodia from man to man, under favorable conditions, has been entirely disproved, for the amount of experimental work on this phase of malaria is surprisingly small and not sufficient to disprove definitely possible mechanical transmission under favorable circumstances. Malarial paroxysms may be produced in man by the intravenous or subcutaneous injection of comparatively small amounts of blood (much less than 1 cc.), and it is not beyond the range of possibility that biting insects, such as mosquitoes, bedbugs, etc., might directly transfer the infection to very susceptible persons through regurgitation in biting, after an interrupted feed on a patient whose blood contained large numbers of plasmodia, or by the blood remaining on, or in, the biting parts. While this method of transmission is merely theoretical at present and perhaps somewhat chimerical, it would explain the exceedingly explosive epidemics of malaria that sometimes occur, as the recent terrible epidemics in Russia, in which the rapid progress of the infection is difficult to explain by the ordinary method ot transmission which requires several days of development of the plasmodia in the mosquito before the insect can infect man. At any rate, it would be wise to investigate this subject carefully, for so little has been done experimentally in this direction that it cannot be stated truthfully that the question has been scientifically answered. To scoff at this method of transmission is not to prove that it is impossible, and the recent developments in the knowledge of the transmission of yellow fever contributed by the Rockefeller Yellow Fever Commission in Africa should make one very hesitant in accepting a thing as disproved unless much experimental work has definitely proved it to be an impossibility. There is no question that certain infections produced by trypanosomes, as surra, are conveyed directly from animal to animal by the fly mechanically transmitting the infection, although, in addition, a cycle of development of the trypanosomes may occur within the same fly which

renders it infective for long periods of time, and it is possible, although not probable, that this type of transmission may also occur in malaria under exceptionally favorable conditions.

While the malaria plasmodia are transmitted from man to man after undergoing a cycle of development in mosquitoes, it is only in certain mosquitoes that this cycle of development is possible, and these all belong to the genus *Anopheles,* so far as has been ascertained. It is true that malaria does not occur where mosquitoes belonging to the genus *Anopheles* are absent, even though plenty of persons having gametocytes of the plasmodia in their blood are constantly present. One of the best examples of the truth of this statement is afforded by the Hawaiian Islands. Although a careful search has been made in these islands by trained entomologists for many years for mosquitoes belonging to the genus *Anopheles,* none has ever been found, and it is well known that no instance of malaria originating in these islands has ever been placed on record, despite the fact that the malarial infections are being constantly introduced into this locality, especially into Honolulu, in the persons of "carriers" of the plasmodia, both civilian and military. In the military hospitals in Honolulu, one constantly see cases of malaria, but these are invariably relapses of an infection acquired elsewhere and there is no record of a case of malaria originating in Honolulu, or in any other portion of the territory of Hawaii, so far as I am aware. In other words, it is impossible for malaria to be transmitted in this locality because of the absence of mosquitoes belonging to the genus *Anopheles,* although other mosquitoes are numerous at all times. This instance of freedom from malaria is also a conclusive argument against the possibility of the direct mechanical transmission of the aforementioned infection, but does not prove that such a method may not occur under more favorable conditions.

*Factors Influencing the Transmission of the Malaria Plasmodia.*—There are many factors which influence the transmission of the malaria plasmodia which must be understood if one is to have a clear conception of the epidemiology of malaria. These factors concern both man and the mosquitoes transmitting the plasmodia, and explain what often appear to be unexplainable phenomena connected with the diffusion of these infections. Thus, it does not always follow that because persons infected with the plasmodia and mosquitoes capable of transmitting them are present in a locality the locality is necessarily very malarial, for both infected man and mosquitoes may be present with a comparatively small amount of malarial infection originating in the locality, as there are so many factors which influence the transmission of the infection. In order that mosquitoes become infected with the

malaria plasmodia, there must be a sufficient number of human beings present in whose blood gametocytes of the plasmodia occur, in sufficient numbers to infect, and in order that the infection may be transmitted to man by the mosquito, the gametocytes must develop to maturity in the mosquito and the insect must bite a nonimmune person. This sequence of events may be interrupted at several points and the transmission of the plasmodia rendered impossible. In nature, such interruptions occur frequently, and when such interruptions do occur, malaria is mildly endemic and only sporadic cases occur, while if such interruptions do not occur and all conditions are favorable to the transmission of the plasmodia, real epidemics of malaria may occur, or the cases of infection will be numerous.

The factors influencing the transmission of the malaria plasmodia are divided into three groups, as follows: factors concerning the development of the plasmodia in man; those concerning the development of the plasmodia in the mosquito; and those concerning the recipient of the plasmodia from the mosquito.

*Factors Concerning the Development of the Plasmodia in Man.*—As already mentioned, in order for the transmission of the plasmodia of malaria to the mosquito to be successful, there must be present persons whose peripheral blood contains gametocytes, i.e., so-called "carriers" of the infection. In addition, there must be enough of these "carriers" present to infect a considerable number of mosquitoes, for if only one or two "carriers" are present, few mosquitoes will become infected, and the number of malarial infections in that locality will be small.

The percentage of persons showing gametocytes in their blood varies greatly in different localities, but from 70 to 80 per cent of patients suffering from malaria will probably become "carriers," i.e., have gametocytes in their blood, if they are untreated or improperly treated. In infections with *Plasmodium falciparum*, the estivo-autumnal plasmodium, I [84] found that 80 per cent of Filipinos having recurrent infections showed gametocytes in their blood, while only about 33 per cent of the infections in American soldiers resulted in the production of gametocytes. The smaller percentage in American soldiers was undoubtedly due to earlier and more thorough treatment, as little if any treatment had been given the Filipinos who were studied. I have found that about 50 per cent of patients admitted to hospital with recurrent infections with *Plasmodium vivax* and *Plasmodium malariae*, the tertian and quartan plasmodia, show gametocytes in the peripheral blood, and that these forms almost invariably develop in experimental infections in

84. Craig, C. F. (footnote 59, first reference); (footnote 21, seventh reference).

paretic patients. That a smaller percentage of patients develop gametocytes under some conditions is shown in the study of this subject by Mansfield-Anders [85] in Zanzibar, who examined 1,542 children from 1 to 6 years of age and found 1,047, or 67.8 per cent, with plasmodia in their blood but only 211, or 13.6 per cent, of these showed gametocytes.

Not only must gametocytes be present in the peripheral blood of man in order for the mosquito to become infected, but there must be enough gametocytes present to infect the insect. In 1910, Darling [86] showed experimentally that it is apparently necessary for the peripheral blood of man to contain at least 12 gametocytes per cubic millimeter, or more than 1 in 500 leukocytes, in order to be infective to the mosquito; his observations were confirmed by Thomson.[87] More recent observations, based on knowledge derived from experimental infections with malaria in paretic patients, have shown that less than the number of gametocytes stated by Darling as being necessary to infect mosquitoes may sometimes produce such infection. Thus, St. John [16] was able to infect mosquitoes with blood from malarial patients in whom the gametocytes numbered only 1 in 500 leukocytes, and others have reported successful results with patients whose blood contained less than 1 gametocyte in 500 leukocytes.

The transmission of the plasmodia to the mosquito is further restricted by the fact that in order for the insect to become infected and for the plasmodia to undergo their normal development in the insect, there must be the proper numerical relationship between the male and female gametocytes, i.e., the microgametocytes and the macrogametocytes. Usually the female gametocytes are more numerous than the male, the usual proportion in my experience being three or four females to one male, but sometimes the females are numerous, and prolonged search will show few, if any, males. Under such conditions the infection of the mosquito would be exceptional, and if no males were present, impossible. Knowles,[48] in the malarial infections he studied found the proportion of females to males to be 10 to 1; Abrami and Senevet [88] found it to vary from 2 to 1 to 3 to 1; Stephens and Gordon,[89] 10 to 1; and James,[90] in experimental malaria in paretic patients produced by infected mosqutoes, found the proportion of females to males to be 3 to 1. It will thus be seen that while there is some variation in this respect, the average number of female gametocytes to male gametocytes

85. Mansfield-Anders, W. L.: Tr. Roy. Soc. Trop. Med. & Hyg. **21**:207, 1927.
86. Darling, S. T.: Rep. Isthmian Canal Com., 1910, p. 22.
87. Thomson, J. G.: Ann. Trop. Med. **7**:509, 1918.
88. Abrami, P., and Senevet: Bull. Soc. path. Exot. **8**:167, 1920.
89. Stephens and Gordon: Ann. Trop. Med. **18**:1, 1924.
90. James, S. P.: Tr. Roy. Soc. Trop. Med. & Hyg. **19**:278, 1926.

is 3 to 1, and that when this number is present the mosquito becomes infected. If either female or male gametocytes are absent, infection of the mosquito is, of course, impossible.

In order to infect mosquitoes, the gametocytes in the blood of man must be mature for the immature gametocytes are not infective to mosquitoes. It has repeatedly been shown that gametocytes are not infective to the mosquito, when they first appear in the blood, but that several days must elapse before the insect can be infected by them. Wenyon [64] states that the gametocytes of *Plasmodium vivax* become mature in about four days, and it is generally stated that from four to eight days must elapse after the time of the appearance of gametocytes in the peripheral blood before they are infective to the mosquito. I believe that this problem is far from solved and that much more work should be done on it before one accept as final the few data that are at present available regarding the required age of the gametocytes before they become infective to the mosquito. It is undoubtedly true that the developing gametocyte within the red blood corpuscle is not infective, but just how old the fully developed gametocyte must be before it becomes infective to these insects is still a question.

The same remarks will apply to the question of how long the gametocytes persist in the blood of man and how long they remain infective to the mosquito. Because a person once shows gametocytes in sufficient number in his blood to infect mosquitoes, it does not follow that he remains infective to these insects indefinitely, for it has been conclusively shown that unless the gametocytes are removed from the blood within from nine to twelve days by the mosquito, they will die of old age. Unless the gametocytes are continually replenished by the schizonts which develop these bodies, the individual will soon become noninfective to mosquitoes because all the gametocytes will have perished. This is a most important point to remember in prophylaxis against malaria for it indicates that proper treatment of patients with primary infections, by killing the schizonts, will prevent the development of gametocytes and hence the infection of the mosquito, while the same result will be obtained, although less readily, by proper treatment even after the development of gametocytes.

There is evidence that the presence of quinine in the blood of a person harboring the gametocytes of the malaria plasmodia renders them inert so far as development in the mosquito is concerned. Thus Wenyon [91] demonstrated that anopheles mosquitoes could be infected with *Plasmodium falciparum* from patients taking quinine, but that infection rarely followed in the case of *Plasmodium vivax*, from which he concluded that quinine affected the gametocytes of *P. vivax*, but not

91. Wenyon, C. M.: J. Roy. Army Med. Corps **37**:352, 1921.

those of *P. falciparum*. His results were confirmed by Mayne,[92] and more recently, Barducci [93] has found that patients whose blood contained *Plasmodium vivax, Plasmodium malariae* and *Plasmodium falciparum*, and who were taking quinine, were not infective to *Anopheles maculipennis*, the common transmitter of malaria in Europe, as the gametocytes failed to complete their life cycle in the insects. These observations appear to prove that the gametocytes of the malaria plasmodia are so affected by quinine as to render their normal development in the mosquito impossible, thus preventing the transmission of the infection to man.

It has also been demonstrated by many observers that not all mosquitoes biting patients whose blood contains numerous gametocytes become infective to man. In fact, only a small proportion of the insects biting such patients ever become infective under experimental conditions, a fact which has been proved again and again in the treatment of patients with paresis by the production of malaria by the mosquito.

*Factors Concerning the Development of the Plasmodia in the Mosquito.*—As already stated, the malaria plasmodia can be transmitted from man to man only by mosquitoes belonging to *Anophelinae;* the factors which govern the development of the gametocytes in these mosquitoes are many and various, and exercise an enormous influence on the transmission of malarial infections.

Temperature is a most important factor in the development of the gametocytes of the malaria plasmodia in the mosquito, for unless the temperature is favorable the gametocytes will not develop into sporozoites in the mosquito and thus the transmission of malaria to man will be prevented.

One of the first investigations regarding the relation of temperature to the transmission of malaria by the mosquito was that of Jancso,[94] who determined that the lowest temperature at which the malaria plasmodia would develop in the mosquito was between 15 and 16 C. (59 to 61 F.), but further observations have shown that the plasmodia will develop in the mosquito at much lower temperatures. Wenyon [95] found that temperatures between 9.6 and 18.2 C. (48.5 to 64.5 F.) prevented development, while Thomson and Woodcock [96] state that the optimum temperature for the development of *Plasmodium vivax* in the mosquito is 25 C. (77 F.) and complete development in the mosquito occurs in

92. Mayne, B.: Public Health Rep. **37**:1059, 1922.

93. Barducci, A. V.: Riv. Malariologica **5**:594, 1926.

94. Jancso, N.: Centralbl. f. Bakteriol. **38**:650, 1905.

95. Wenyon, C. M.: Official History of the War, Medical Services, Diseases of the War, London, printed by the British Government.

96. Thomson, J. D., and Woodcock, in Byam and Archibald: Practice of Medicine in the Tropics, New York, Oxford University Press, vol. 2.

eleven days, while for *Plasmodium malariae* the optimum temperature is 22 C. (72 F.) and complete development in the mosquito occurs in from eighteen to twenty-one days. They also determined that the optimum temperature for *Plasmodium falciparum* is 30 C. (86 F.) and complete development occurred in from ten to eleven days. From these observations it will be noted that the various species of plasmodia require different temperatures for development, and this explains, to some extent, the geographical distribution of the various types of the infection. Thus, *Plasmodium falciparum*, the cause of estivo-autumnal, or so-called "tropical malaria," requires a temperature of over 80 F. for its optimum development in the mosquito, which explains its greater prevalence in the tropics and its occurrence in subtropical and temperate regions only during the hot months of summer and early autumn.

A cold temperature prevents the development of the malaria gametocytes in the mosquito, but does not always destroy them. King [97] found that in *Anopheles quadrimaculatus*, the principal transmitter of malaria in the United States, *Plasmodium vivax* resisted a temperature of 0 C. (32 F.) for four days and a temperature between 7 and 8 C. (45 to 47 F.) for seventeen days and that sporozoites developed from the oocysts after exposure to these temperatures, and the mosquitoes became infective. He also found that *Plasmodium falciparum* withstood a temperature of 1.7 C. (35 F.) for twenty-four hours.

The resistance of the plasmodia in the mosquito to low temperatures indicates that, under certain conditions, the infection may persist in these insects throughout a winter, and that man may become infected from them in the spring. That it is possible for malarial infection to be carried over the winter in hibernating mosquitoes has been demonstrated by several observers. Wenyon [98] kept infected mosquitoes at temperatures corresponding to winter temperatures for various periods of time, after which the insects were kept at temperatures favoring the development of the gametocytes, and he found that the winter temperatures to which the insects had been exposed did not prevent the development of the gametocytes when the temperature was again favorable for such development. He showed that the gametocytes in the mosquitoes kept at winter temperatures ceased further development and remained in this condition for weeks and even months, but resumed their normal development when the temperature conditions became favorable. James [15] found that the oocysts and the sporozoites of the malaria plasmodia in the mosquito were not killed in mosquitoes living continuously for three weeks at a temperature between 4 and 5.5 C. (39 to 41.5 F.) or when the temperature for six days was below the freezing point, nor when the

97. King, W. V.: J. Exper. Med. **25**:495, 1917.
98. Wenyon, C. M.: J. Roy. Army Med. Corps **37**:81, 1921.

insects lived intermittently at low and high temperatures for long periods of time. He says:

> We do not doubt that benign tertian malaria (*P. vivax*) can be carried through even a severe winter in hibernating mosquitoes. . . . It seems clear, also, that the carriage of the parasite in the mosquito through the winter may be in either the oocyst stage or the sporozoite stage, or both.

The recent observations of Chodukin and Lisowa [99] confirm those of James. These observers found that the ookinetes of *Plasmodium vivax* can remain in the stomach of *Anopheles elutus,* a common transmitter of malaria, for eight days at a temperature of 2 to 8 C. (35.5 to 46.5 F.) and then develop into oocysts; that the oocysts will survive a temperature of from 0 to 8 C. (32 to 46.5 F.) for thirty days; that sporozoites can live in the salivary glands of mosquitoes for thirty days at temperatures between 0 and 8 C. (32 to 46.5 F.), and they conclude that the winter and spring infections with malaria may be produced by infected hibernating mosquitoes.

From the evidence that has accumulated, I believe that under favorable conditions, malaria plasmodia may be carried throughout a winter in hibernating mosquitoes and that these insects may be responsible for the primary infections occurring in the early spring in certain localities.

Whether mosquitoes once infected with the gametocytes of the malaria plasmodia remain so as long as they live is still undecided, although the evidence is not in favor of such a view. Under favorable conditions the plasmodia remain alive for considerable periods in mosquitoes that are kept in captivity. Thus, Mayne [92] found the plasmodia in mosquitoes kept in captivity for seventy, seventy-one, eighty-three and ninety-two days and that a mosquito infected fifty-five days previously was capable of transmitting the infection to man, but that mosquitoes infected sixty-one, sixty-six and sixty-seven days previously did not transmit the plasmodia.

Hereditary transmission of the plasmodia in the mosquito has not been proved and there is no reason to believe that this ever occurs.

The number of mosquitoes present in any locality that can transmit the infection to man has much to do with the prevalence of malaria provided sufficient human "carriers" of the plasmodia are also present. It is evident that the greater the number of "carriers" and of suitable species of transmitting mosquitoes present, the greater the chance of man becoming infected. While this is so, there must also be a nonimmune population present, or few persons will develop the symptoms of malarial infection. If "carriers" are numerous, a comparatively small number of mosquitoes may cause a great deal of infection, as one

99. Chodukin and Lisowa: Pensee Med. d. Usbekistane. Taschkent; abst. Trop. Dis. Bull. **25**:128, 1927.

infected insect may infect many human beings, and in most malarial localities the percentage of infected mosquitoes is surprisingly small.

The percentage of infected mosquitoes is of great importance in the transmission of malaria to man, and if the percentage is large and there are numerous human "carriers" veritable epidemics of malaria may occur. Usually the percentage of infected mosquitoes is much smaller than one would expect, considering the number of human "carriers" in most malarial localities and the chances for infection of the mosquitoes. Thus, Celli,[100] in Italy, found only 2.5 per cent of the anophelines he examined infected; A. Plehn [101] found only 2.3 per cent of anopheles infected in very malarial regions in Africa, while the Sergents [102] found only 1.6 per cent infected in Algeria. Swellengrebel [103] dissected 10,873 anopheline mosquitoes in Amsterdam and found that the rate of infection in 1922 was 5.7 per cent and in 1923 was 6.2 per cent, while Mansfield-Anders [85] examined 3,000 anopheline mosquitoes in Zanzibar and found 7.1 per cent infected. While the figures given indicate the usual percentages of infected mosquitoes found in malarial regions, much higher percentages are observed in some regions. Thus, Vestschezerow [104] examined 194 *Anopheles maculipennis* captured in sleeping quarters in the Caucasian region of the Black Sea and found 20 per cent infected with the malaria plasmodia. I [105] found as high as 35 per cent of anophelines infected at Camp Stotsenburg, P. I., during the height of the malarial season, these insects being caught in sleeping quarters and around the hospital. Experimental infection rates in mosquitoes as high as 80 per cent have been reported by Wenyon, James and others, but it is doubtful if such high rates ever occur under natural conditions.

The relation of the number of feedings by the mosquito has much to do with the percentage of infection of these insects. Numerous observers, especially Yorke [106] and James [90] have shown that a single feeding of a mosquito on a good "carrier" seldom results in the infection of the mosquito, but that three or four feedings on the same person by the same species of mosquito results in a high percentage of infections. Thus James found that 40 per cent of his experimental mosquitoes became infective after two feedings, while from the third to the fifth

100. Celli, A.: Tr. Int. Cong. Hyg. & Demog., Washington, D. C., 1913, vol. 5, p. 526.

101. Plehn, A.: Arch. f. Hyg. **59**:1, 1904.

102. Sergent, E., and Sergent, E.: Atti. d. Soc. p. g. studi d. malaria **10**:217, 1909.

103. Swellengrebel, N. H.: Nederl. Tijdschr. v. Geneesk. **68**:750, 1924.

104. Vestschezerow, N. I.: Rev. Microbiol. et Epidem. **5**:233, 1926.

105. Craig, C. F. (footnote 59, first reference).

106. Yorke, W.: Tr. Roy. Soc. Trop. Med. & Hyg. **19**:108, 1926.

feeding 70 per cent of the insects were infected. It is probable that in nature only a small number of mosquitoes become infected after one feeding and it is also true that even after repeated feedings some mosquitoes do not become infected, especially if fed on some persons, as it has been proved that certain persons, whose blood may contain many gametocytes, are poor infectors of mosquitoes.

The particular species of anopheline mosquitoes present in a locality has much to do with the prevalence of malarial infection, for while only species of mosquitoes belonging to *Anophelinae* can transmit malaria, many species belonging to this family cannot transmit the plasmodia, while others are seldom active under natural conditions in the transmission of the parasites. This fact is becoming more and more important in prophylaxis, as comparatively few species are natural transmitters of malaria and time and expense can be saved in prophylaxis by directing preventive measures only toward the species that are known to be active in this respect. Probably the first to call attention to this fact were Stephens and Christophers,[107] in India, who dissected 328 *Anopheles culicifacies* and found 5.4 per cent showing sporozoites of the malaria plasmodia, while 879 *Anopheles rossi* dissected by them did not show a single infection with the malaria plasmodia. Their observations showed conclusively that *Anopheles rossi* could be ignored as a transmitting agent of malaria in India, but that *Anopheles culicifacies* was an important transmitter of these infections. Their observations have been confirmed by numerous investigators and it has been demonstrated that *Anopheles rossi* seldom, if ever, transmits malaria in nature, although it may be experimentally infected. Many species of anopheles cannot even be experimentally infected with the malaria plasmodia, so that it is possible to have anopheles mosquitoes in a locality without malaria. It is known that, under natural conditions, comparatively few of the many species of anopheline mosquitoes act as transmitters of the malaria plasmodia and that these species are often confined to certain regions because of their biologic requirements. Thus, in Europe, *Anopheles maculipennis* is the most common transmitter of malaria; in the United States, *Anopheles quadrimaculatus;* in Panama, *Anopheles albimanus;* in Porto Rico, *Anopheles albimanus,* and in the Philippine Islands, *Anopheles ludlowi.*

Darling,[108] by experimenting with *Anopheles albimanus, Anopheles pseudopunctipennis* and *Anopheles malefactor,* and by allowing the insects to feed on patients whose blood contained gametocytes of *Plasmodium falciparum* and *Plasmodium vivax,* found that 70.8 per cent of *Anopheles*

107. Stephens and Christophers: Practical Study of Malaria, New York, Longmans, Green & Company, 1904.

108. Darling, S. T.: Transmission of Malarial Fever in the Canal Zone by Anopheles Mosquitoes, J. A. M. A. **53**:2051 (Dec. 18) 1909.

*albimanus* became infected with plasmodia; 12.9 per cent of *Anopheles pseudopunctipennis,* and none of *Anopheles malefactor.*

Not only is it true that certain species of anopheles mosquitoes are unable to act as natural hosts of the malaria plasmodia, but it is also true that, of the species that act as natural hosts, some are unable to act as such for certain species of the plasmodia while suitable for other species. Thus, several observers in Japan have shown that *Anopheles cohaesus* and *Anopheles formosaensis* are efficient transmitters of the estivo-autumnal plasmodium, *Plasmodium falciparum,* while *Anopheles jesoensis* acts as the transmitting agent of both the tertian plasmodium (*Plasmodium vivax*) and the estivo-autumnal plasmodium. Mitzmain[109] has shown, in the United States, that *Anopheles quadrimaculatus* is an efficient host of *Plasmodium vivax, Plasmodium malariae* and *Plasmodium falciparum,* while *Anopheles punctipennis* and *Anopheles crucians* are efficient hosts of *Plasmodium vivax* and *Plasmodium falciparum* but not of *Plasmodium malariae.* Although Mitzmain has shown that *Anopheles crucians* can be experimentally infected with these plasmodia it is doubtful if this species acts as a transmitter of malaria under natural conditions. Mayne,[110] in a malaria survey of the Okefenoke Swamp, between Georgia and Florida, did not find any malaria, the blood of 347 of the inhabitants being negative for the plasmodia; he also found that the only anopheline mosquito present was *Anopheles crucians.* Of 10,810 specimens of this insect dissected and examined, not one showed infection with the malaria plasmodia, although in the surrounding localities malaria was present and *Anopheles quadrimaculatus* acted as the transmitting agent. Wherever *Anopheles crucians* occurred alone, malaria was not present.

In Formosa, Kinoshita[111] determined that while *Plasmodium falciparum* developed normally in *Anopheles listoni,* it failed to do so in *Anopheles sinensis,* although in the latter species of mosquito *Plasmodium vivax* developed readily. Swellengrebel and his co-workers[112] found that while 100 per cent of *Anopheles ludlowi* became infected experimentally with *Plasmodium falciparum* and 80 per cent with *Plasmodium vivax,* only 4.7 per cent of 107 mosquitoes of this species became infected with *Plasmodium malariae.* In their experience with *Anopheles umbrosus,* they found that none of 87 insects became infected with *Plasmodium falciparum,* but that 5 per cent of 104 insects of this species

109. Mitzmain, M. B.: Pub. Health Rep. **32**:1081, 1917.

110. Mayne, B.: Pub. Health Rep. **41**:1662, 1926.

111. Kinoshita, K.: Arch. f. Schiffs- u. Tropen- Hyg. **10**:621, 676, 708 and 741, 1906.

112. Swellengrebel; Schüffner and Swellengrebel de Graaf: Meded. Burgerlizk. Geneesk. Dienst. Med-Indie **3**:1, 1919.

became infected with *Plasmodium vivax*. Much more evidence could be quoted showing that certain species of anopheline mosquitoes are efficient hosts for certain species of malaria plasmodia and not for others, and this explains, to a certain extent, the relative frequency of the various malaria plasmodia. Thus, *Plasmodium malariae,* the least common of the three well known species of plasmodia of man, develops poorly in almost all species of anopheline mosquitoes which are good hosts for *Plasmodium vivax* and *Plasmodium falciparum;* this undoubtedly has much to do with the comparative rarity of this type of malaria.

It sometimes happens that a species of mosquito, usually regarded as a poor transmitter of malaria, may be the most common transmitter in certain localities. An interesting instance of this has been recorded recently by Davis [113] who found that in the northern part of the Argentine Republic, in the Province of Tecuman, the most prevalent mosquito is *Anopheles pseudopunctipennis* and that it is also the principal transmitter of malaria in this region. This species is known to be of only secondary importance as a transmitter of these infections in most malarial regions in which it occurs, but in this particular locality Davis states that it is solely responsible for the transmission of malaria.

The relation of rainfall and humidity to the transmission of malaria is most important and within recent years much more attention has been paid to this relationship than formerly. The amount of rainfall and humidity influences the reproduction and activities of the transmitting mosquitoes but, in addition to this, humidity has a definite influence on the development of the plasmodia in the mosquito. If mosquitoes are allowed to bite persons whose blood contains gametocytes and these insects are then kept in a dry atmosphere in which there is no water vapor, the gametocytes either do not develop into oocysts in the insects or the oocysts do not produce sporozoites. A certain degree of humidity is necessary for the normal development of the malaria plasmodia in the transmitting mosquitoes, but little is known regarding this subject and it deserves more careful consideration.

In all parts of the world it has been noted that there is a definite relationship between the annual rainfall and the amount of malaria in a locality. Rogers[114] was one of the first to call attention to this relationship and was able to show that a high rainfall caused epidemics of malaria in certain portions of India, although in other portions, as in Eastern Bengal, a deficient rainfall was associated with increased malaria, because the flood waters subsided earlier and allowed a longer period for mosquito breeding and infection. More recently, Gill [115] has

113. Davis, N. C.: Am. J. Trop. Med. **7**:167, 1927.

114. Rogers, L., quoted in Rogers: Recent Advances in Tropical Medicine, Philadelphia, P. Blakiston's Son & Company, 1928, p. 60.

115. Gill, C. A.: Indian J. M. Research **11**:511, 1923.

carefully studied this subject and for several years has been able to issue annual forecasts of malarial epidemics based on the rainfall in certain parts of India. In a recent valuable paper, Kligler,[116] in discussing the epidemiology of malaria in Palestine, shows that the distribution of rainfall and the prevailing temperatures modify the intensity and recurrence of malaria epidemics to such an extent that it is possible to predict the time and probability of an outbreak of these infections with practical certainty, and that such knowledge is of the greatest importance in the prophylaxis against malaria.

While a high annual rainfall is usually favorable for the transmission of malaria by the mosquito, it has been found that in certain localities a low rainfall is more favorable. Thus, Strickland[117] noted that in the Darjeeling Hill tea districts in India, the malarial season extends between April and July, during which time it is dry and warm, but that later the heavy monsoon rains wash away the stream breeding anopheles, as *Anopheles maculatus* and *Anopheles wilmori*, the principal mosquitoes transmitting malaria in this region, and the malaria index falls markedly.

The habits of anopheles mosquitoes have an important bearing on transmission, for the species that live in most intimate contact with man are those most dangerous from the standpoint of the transmission of the plasmodia. Some of the mosquitoes transmitting malaria breed in close proximity to human habitations and these species are usually efficient transmitters of the plasmodia, both because of the chances of their infection from man and because of their suitability for the development of the plasmodia.

*Anopheles maculipennis*, in Europe, and *Anopheles quadrimaculatus*, in the United States, are instances of what may be called "domesticated mosquitoes," living in close contact with man and acting as the principal transmitting agents of the malarial fevers in these localities. In Palestine, *Anopheles bifurcatus* breeds in cisterns and in small collections of water in jars and other receptacles in the houses, and in this region this mosquito is an efficient transmitter of the plasmodia, although in many other regions in which its habits are different, it is of little importance in the transmission of malaria, for it is usually a species living in the open and in the forest and not in close contact with man.

The factors that have been mentioned as influencing the development of the malaria plasmodia in the mosquito and the transmission of these plasmodia to man are those that are best known, but it is probable that many important factors in this respect are still unknown, and it is certain that further research and study will increase the knowledge concerning this subject.

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116. Kligler, I. J.: Am. J. Trop. Med. **8**:183, 1928.
117. Strickland, C.: Indian M. Gaz. **58**:805, 1923.

*Factors Concerning the Recipient of the Plasmodia.*—It is well known that not all persons bitten by mosquitoes which are infective will develop malaria, and this fact has been proved both clinically and experimentally in numerous instances. It follows, therefore, that some persons are resistant or immune to malaria, and that the transmission of malarial infections to such persons is impossible. It is in influencing the resistance or immunity of persons that the economic condition of a people is of much importance in the transmission of malaria. Where much poverty is present, with its attendant lack of food and of suitable shelter, the resistance of the people is lowered and they are more susceptible to malarial infection. While it may be stated correctly that no race of people is immune to malaria, it is true that a natural immunity to these infections does exist in certain persons, which may be broken down under exposure or mental or physical stress, and that so far as the development of symptoms is concerned, some races are less susceptible to these infections than others.

That this immunity to malarial infection may be a family characteristic, is shown by the instances reported by Celli [118] and by Marchifava and Bignami.[119] Celli described four persons living in the Pontine Marshes, a most malarial portion of Italy, who had resided there for many years, had never taken any precautions against malaria, yet who had never suffered from the disease and whose blood was negative for the malaria plasmodia whenever examined. Marchiafava and Bignami tell of a family living in one of the worst malarial regions of the Roman Campagna, in which the grandfather, father and two sons, although continually exposed to infected mosquitoes, had never had malaria, although practically every one else living in the locality suffered from the disease. I have observed many instances of natural immunity in the persons of officers and soldiers of the United States Army serving in Cuba and the Philippines. These persons, although serving in very malarial regions and taking no precautions against infection, never developed malaria although their comrades in the same locality suffered from the infection.

The existence of indiviudal natural immunity to malaria has been experimentally confirmed in the work of various authorities on the treatment of patients with paresis by the induction of malarial infection. It has been shown repeatedly that only a fraction of all persons bitten by experimentally infected mosquitoes develop malaria. Thus, James [120] found that of 221 paretic subjects bitten by known infective mosquitoes, 52 failed to develop the disease after one biting, but some of these became

118. Celli, A.: Malaria, London, Longmans, Green & Company, 1900.
119. Marchiafava and Bignami (footnote 20, second reference).
120. James, S. P.: Tr. Roy. Soc. Trop. Med. & Hyg. **20**:153, 1926.

infected after repeated bitings by the mosquitoes. His experience has been confirmed by that of others.

From experimental evidence that has accumulated regarding the cause of natural immunity in malaria, it would appear that it must be due to immune substances present in the blood of the person who has been inoculated, which prevent the development of the plasmodia, in other words, to antiplasmodial substances. In 1909, I [121] stated that natural immunity in malaria probably depended on antiplasmodial and antitoxic substances in the blood serum, on phagocytosis, and on the possible liberation of antiplasmodial and antitoxic substances from the phagocytes by phagolysis. James [120] believes that it is due to some "unusual chemical or other condition, as yet unknown, in the blood or tissues of certain persons" and that the administration of quinine, by killing a certain number of the plasmodia in an infected person, causes the formation of antibodies which result in a cure of the infection.

It has also been determined that certain persons do not become infected with malaria even after the direct inoculation of blood containing numerous plasmodia. Numerous instances of this kind are on record, and Epstein and Rubinstein [122] describe a case in which four injections of malarial blood failed to produce an infection.

While natural immunity is comparatively rare in malaria and is never a general or racial characteristic, a relative immunity to malarial infection is observed in many native races living in highly endemic regions. Long residence and repeated infection with the plasmodia will bring about a relative immunity to malaria, and in the endemic centers of these infections a blood examination always shows a large amount of latent infection. Usually, the immunity in these people is not an immunity to the infection of the blood by the plasmodia but to the effects of the toxins produced by the development of the plasmodia in the blood. Infection is not prevented, but the symptoms of infection. The subject of latent malaria will be considered in the discussion of the prophylaxis against this disease, as it is of great importance in this respect.

Some recent observations of James, Nicol and Shute [123] (1927) are most interesting and valuable as they indicate that the immunity produced by repeated infections with malaria plasmodia may not only be an immunity to the effects of the plasmodia but also to the plasmodia, and even to specific strains of the plasmodia. In treating paretic patients, by inducing an attack of malaria by experimental infection through the

121. Craig, C. F. (footnote 21, third reference).

122. Epstein and Rubinstein: Beihefte z. Arch. f. Schiffs- u. Tropen- Hyg. **29**:76, 1925.

123. James; Nicol, and Shute: Abst. Papers 7th Cong. Far Eastern Med. Assoc., Calcutta, 1927.

bites of mosquitoes, they found that repeated inoculations by the mosquito produced an immunity to a particular strain of the plasmodium. When a certain strain of *Plasmodium vivax* was used, the attack produced by the first inoculation by the mosquito was normal in severity and symptomatology and had to be terminated therapeutically; the attack produced by the second inoculation by the insect usually died out spontaneously; and after the third inoculation by the mosquito, infection failed to result or an infection was produced unaccompanied by symptoms and with only a few plasmodia present in the peripheral blood. This immunity was found to be confined apparently to the particular strain of *Plasmodium vivax* used for the experiments, as another strain of the same species, when inoculated by the mosquito, produced infection and the development of symptoms after the usual incubation period. It was noted, however, that the attacks produced by the new strain died out spontaneously, so that a slight immunity to the new strain must have been produced.

The recent observations on complement fixation in malaria by Thomson,[124] Horowitz and Wlassowa,[125] Kingsbury [126] and others, and those on the presence of precipitins in the blood of malarial patients by Taliaferro and Fisher [127] and Taliaferro and Taliaferro,[128] which are discussed in the consideration of the diagnosis of malaria, prove beyond question that antibodies to the malaria plasmodia do exist in the blood and are produced in response to infection with the parasites. This fact has been taken advantage of by Kauders [129] in an effort to produce artificial immunity and cure through the injection of the blood serum of patients who have recovered from an attack of the disease. He found that a series of from seven to nine injections of the serum before malarial blood was injected did not prevent infection and the incubation period was shortened, but in a third series of experiments malarial blood was injected and also eight injections of serum from a patient who had recovered from malaria. In one such patient, twelve subsequent injections with blood containing malaria plasmodia did not result in infection and no symptoms were observed during five months of observation. In twelve patients having malaria, Kauders gave from one to two injections of from 1 to 2 cc. of the blood serum of a patient in whom infection had disappeared, so-called "refractory serum," with the following results:

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124. Thomson, J. G.: Observations on Malaria by Medical Officers and Others, London, 1919.

125. Horowitz and Wlassowa: Ztschr. f. Immunitat. **40**:268, 1924.

126. Kingsbury, A. N.: Tr. Roy. Soc. Trop. Med. & Hyg. **21**:233, 1927.

127. Taliaferro, W. H.; Taliaferro and Fisher: J. Prev. Med. **1**:343, 1927.

128. Taliaferro, W. H., and Taliaferro: J. Prev. Med. **2**:147, 1928.

129. Kauders, O.: Centralbl. f. Bakteriol. **104**:158, 1927.

In three there was no effect, but in nine there was a marked effect, the fever disappearing and failing to return. The blood serum of normal persons, or those suffering from acute symptoms of malaria, had no effect when used in this manner.

It has been shown experimentally that by repeated passage through man of *Plasmodium vivax,* the tertian plasmodium, races that originally produced gametocytes ceased to do so, thus becoming sterile so far as the infection of mosquitoes is concerned. This change must be brought about by the action of substances in the recipient's blood which prevent the production of the sexual forms. Cuboni [130] studied the two strains of *Plasmodium vivax* which are used at Wagner-Jauregg's clinic in the treatment of patients with paresis. At the beginning of the study, these two strains produced gametocytes in the blood of the inoculated patients, but after 134 and 143 direct passages, respectively, gametocytes were not produced, although schizonts were always to be found without difficulty. Whether the same thing occurs after natural inoculation by the mosquito has not been determined.

The presence of quinine, in therapeutic doses, in the blood of the recipient, while it will not prevent infection in many instances, does prevent the development of gametocytes and such persons will not be infective to mosquitoes. This subject will be considered more extensively in the consideration of the use of quinine in prophylaxis against malaria. Even though the amount of quinine is not sufficient to prevent the origin of gametocytes, the sexual forms produced do not undergo normal development in the mosquito, as has already been noted, and the insect does not become infective.

*Experimental Infection of Lower Animals.*—There is only one instance of the successful transmission of a malarial plasmodium of man to any lower animal, that of the infection of a chimpanzee with *Plasmodium vivax* by Mesnil and Roubaud,[131] already noted. Deaderick [132] stated that the following lower animals have been inoculated with blood containing the malaria plasmodia without results: horses, mules, dogs, monkeys, rabbits, mice, guinea-pigs, hedge-hogs, wolves, cats, bats, pigeons, doves, magpies, screech-owls, turtles, frogs and lizards. Bass,[133] in three different sets of experiments, inoculated four guinea-pigs, five rabbits and one monkey (*Macacus rhesus*) intravenously with blood containing *Plasmodium falciparum,* but the animals remained apparently well and repeated examination of the blood extending beyond the incuba-

130. Cuboni, E.: Wien. klin. Wchnschr. **39**:1475, 1926.

131. Mesnil and Roubaud: C. R. Acad. Sc. **165**:39, 1917.

132. Deaderick, W. H.: A Practical Study of Malaria, Philadelphia, W. B. Saunders & Company, 1909.

133. Bass, C. C.: Am. J. Trop. Med. **2**:107, 1922.

tion period of malaria in man failed to show plasmodia except in one guinea-pig twenty-four hours after inoculation. In this case, the plasmodia had not developed although in cultures of the injected blood the plasmodia had all grown beyond the ring-stage found in the inoculated animal. The plasmodia were proved to be viable at the time injected, by cultivation, and it was also found that cultures made in blood serum from the several animals failed to develop although those in cultures made at the same time in human serum developed in a normal manner. More recently, Yoshino [134] claims to have found "ring-forms" of *Plasmodium vivax* in the blood of young guinea-pigs, rabbits and dogs for from two to eight days after the subcutaneous injection of blood containing the parasite. Gametocytes were not seen or larger developmental forms, but he claims to have been successful in transmitting the infection from man to the guinea-pig and from guinea-pig to guinea-pig. Yoshino's observations have not been confirmed.

*Incidence of Malarial Infection.*—The incidence of malarial infection varies greatly in different localities, due to the factors that have been mentioned. In some regions in which malaria is endemic only, a few cases occur during the malarial season while in others the vast majority of the inhabitants suffer from the infection. The incidence is always greater in the tropical and subtropical regions than in temperate regions, except under exceptional conditions which favor the development of epidemics of the disease.

The general incidence of malaria is discussed more fully in the section of this review dealing with latent malarial infection and its relation to prophylaxis. The incidence of the various malaria plasmodia in different regions will be considered briefly here.

In temperate and subtropical regions and in some portions of the tropics, *Plasmodium vivax* is the most common species of malaria plasmodium encountered, and the infections so produced are almost invariably benign, hence the name, benign tertian malaria. In Baltimore, Thayer and Hewetson [135] observed 542 cases of malaria of which 338 were caused by *Plasmodium vivax,* and of the numerous cases studied by Mannaberg,[18] in Vienna, over 80 per cent were due to this parasite. Mathis and Leger [136] found that of 344 malarial infections studied in Tonkin, 190, or 55 per cent, were caused by this plasmodium, while it is well known that a large proportion of the malarial infections observed in the United States, north of the Gulf States and in California, are due to *Plasmodium vivax*. Even in some tropical regions,

134. Yoshino, M.: Arch. f. Schiffs- u. Tropen- Hyg. **30**:624, 1926.

135. Thayer and Hewetson: The Malarial Fevers of Baltimore, Johns Hopkins Hosp. Rep., 1895.

136. Mathis and Leger: Recherches de parasitologie et de humaines et animales au Tonkin, Paris, 1911.

however, infections with this plasmodium are more numerous than with *Plasmodium falciparum,* although in most tropical regions the latter plasmodium is the most common cause of malaria, the infections with *Plasmodium vivax* not causing more than from 25 to 30 per cent of the total malarial infections present. Thus Mühlens found that of 2,107 malarial cases examined in Jerusalem, only 593, or 28.6 per cent, were due to *Plasmodium vivax,* and in some tropical regions this plasmodium is rarely observed, as shown by Leger and Baury,[137] who did not find infections with this plasmodium among the natives of Guinea, East Africa, and only nine infections with *Plasmodium vivax* in 155 cases of malaria observed at Dakar, East Africa.

A considerable number of the inhabitants of localities in which tertian malaria is endemic will show *Plasmodium vivax* in the peripheral blood although they may not have any symptoms of the infection, as well be noted later. The incidence of infections with *Plasmodium vivax* increases as the temperate zones are approached, and in most regions in these zones it is the prevailing type of malarial infection.

The incidence of infection with *Plasmodium malariae* is almost invariably small in localities in which it is endemic, and there are some regions in which this species never occurs, so far as can be ascertained. It is by all odds the most rare of the three well known species of human malaria plasmodia as is shown by table 1 compiled from various authorities:

TABLE 1.—*Infections with Plasmodium Malariae*

| Observer | Locality | Cases of Malaria Observed | Infections with P. malariae |
|---|---|---|---|
| Davis and Boyd | Rio de Janeiro (state) | 1,700 | 20 |
| Pinot | Blida | 4,211 | 26 |
| Mailiot | Algeria | 2,338 | 21 |
| Laveran | Algiers | 311 | 7 |
| Thayer and Hewetson | Baltimore | 1,680 | 15 |
| Griesinger | Tubingen | 414 | 3 |
| Mannaberg | Vienna | 144 | 8 |
| Craig | Cuba, Philippines and United States | 5,000 | 38 |

This table demonstrates that infection with *Plasmodium malariae* is rare in the experience of the observers mentioned, but it may occur much more frequently in certain regions. Thus Leger and Baury [137] in Guinea, East Africa, examined ninety-six natives and found *Plasmodium malariae* in the blood of thirty-eight, and *Plasmodium falciparum* in the blood of only twenty-seven, thus proving that the quartan plasmodium was the most common species in that particular locality. The same observers examined the blood of 366 natives at Dakar, East Africa, 155 of whom showed malaria plasmodia; they found 42, or 27 per cent, infected with *Plasmodium malariae,* while 104, or 67 per cent, were infected with *Plasmodium falciparum,* and only 9, or 5.8 per cent, with

137. Leger and Baury: Bull. Soc. path. Exot. **15**:497, 1922.

*Plasmodium vivax*. It is thus evident that while *Plasmodium malariae* is usually a rare species, there are regions in which it causes much of the malaria present and it may, indeed, be the prevailing species and the most frequent cause of malaria.

The incidence of infections with *Plasmodium falciparum* is greatest in the tropical and subtropical regions and least in temperate regions. This species is the prevailing one in most tropical regions and in many subtropical localities, causing from 50 to 80 per cent of the malarial fevers, but in some subtropical regions and in the temperate zones infection with *Plasmodium vivax* is more common. Infections with this plasmodium are sometimes called malignant tertian infections to distinguish them from infections caused by *Plasmodium vivax*, as these are the infections that furnish most of the fatal cases of malaria.

In the United States, the Gulf States, the Mississippi Valley and the Sacramento and San Joaquin Valleys in California are endemic centers of infection with *Plasmodium falciparum*, while sporadic cases occur in many other localities. In an examination of the blood of over 2,000 soldiers returning from the Philippine Islands infected with malaria, I found no less than 1,662, or 30.3 per cent, infected with *Plasmodium falciparum*, and at Camp Stotsenberg, on the Island of Luzon, in 1905, no less than 75 per cent of the malarial infections were caused by this parasite. In many regions in the tropics, the percentage of infection with this species is lower than those recorded, for in East Africa, Leger and Baury found only 47 per cent of malarial infections to be due to this species, and in Jerusalem, Mühlens found that only 988, or 47.7 per cent of 2,071 cases of malaria that he observed were caused by *Plasmodium falciparum*.

Latent infection with this plasmodium is common in all endemic regions.

## SYMPTOMATOLOGY

*Period of Incubation.*—The period of incubation of malaria caused by the different species of plasmodia has been definitely determined for both natural and experimental infections, and within recent years much interesting data have accumulated regarding this subject. It has been found that the period of incubation is different for infection brought about by the direct inoculation of blood containing the plasmodia and by the bites of infected mosquitoes, and that it varies considerably in experimental and natural infections and with the different species of plasmodia.

The period of incubation after infection with *Plasmodium vivax* brought about by direct inoculation of blood containing this plasmodium varies considerably. Antolisei and Angelini,[138] who first produced

138. Antolisei and Angelini: Riforma Med. **5**:1352, 1358, 1889.

malaria in this manner with this plasmodium, found the incubation period to be eleven days, while Bein [139] found it to be twelve days, Bacelli,[140] six days and Mannaberg,[18] twenty-one days. These early observers thus showed the period to vary from six to twenty-one days after the direct inoculation of blood containing the plasmodium, the average period being from eleven to twelve days.

These observations have been greatly extended since the prevalent method of treatment of patients with paresis by the production of malaria by inoculating blood containing *Plasmodium vivax* has been practiced. Reese and Peter [141] found the incubation period to vary from five to eight days after intravenous injection of blood containing the parasites and from ten to twenty-five days after subcutaneous injection of infected blood. Dattner and Kauders,[142] using intravenous injections, found the period of incubation to vary from four to seven days, and subcutaneous injection, from six to ten days. The number of plasmodia injected probably accounted for the differences noted in these different cases. Mühlens and Kirschbaum,[12] however, found that the period of incubation varied greatly even in patients inoculated at the same time, in the same manner, and with the same amounts of infected blood, thus demonstrating the varying resistance to the infection of different persons. St. John [143] found that after subcutaneous inoculation of blood containing *Plasmodium vivax* the incubation period varies from seven to twenty days, the average period being slightly over twelve days.

The period of incubation after the bites of infected mosquitoes was stated to be from four to twenty-four days by the older experimenters, who first proved the relation of certaian anophelines to the transmission of malaria in this manner. Thus Bastianelli and Bignami [144] found the incubation period to be eighteen days, while Schüffner [145] found it to be sixteen days. More recent data obtained in the experimental production of malaria in paretic persons by the bites of known infective mosquitoes have shown that there is great variation in the period of incubation of tertian malaria produced in this manner. Thus Yorke [146] found that the incubation period from the bites of the infected mosquitoes to the first rise in temperature varied from nine to twenty-two days, while the first plasmodia were first seen in the blood in from thirteen to twenty-

139. Bein, A.: Charité-Ann., 1891, p. 181.

140. Baccelli, A.: Studien über Malaria, Berlin, S. Karger, 1895

141. Reese and Peter: Med. Klin. **20**:372, 410, 1924.

142. Dattner and Kauders: Klinische und experimentelle Studien sur therapeutischen Impf-Malaria, Leipzig and Vienna, 1924.

143. St. John, J. H.: Am. J. Syphilis. **11**:14, 1927.

144. Bastianelli and Bignami: Ann. d'Igiene **9**:245, 1899; Bull. d. r. Acad. med. di Roma, 1899, part 3, vol. 26.

145. Schüffner, W.: Arch. f. klin. Med. **64**:428, 1899.

146. Yorke, W.: Lancet, 427, 1926.

one days. James,[147] in his work on paretic patients, found that the period of incubation after the bites of mosquitoes infected with *Plasmodium vivax* was most often twelve days but almost as often it was ten days. The longest period he noted was twenty-three days and the shortest was seven days. Ninety per cent of his patients had fever before the seventeenth day after the mosquitoes bit, and nearly 70 per cent between the tenth and seventeenth days. Usually plasmodia were not found in the blood until the second or third day after the primary rise in temperature. His figures were based on 109 cases of *Plasmodium vivax* infection in persons with pareses.

The period of incubation of *Plasmodium vivax* infections under natural conditions is obviously difficult to ascertain, but the data at hand shows that it is practically the same as after experimental production of the infection by the bites of infective mosquitoes. That it varies exceedingly under natural conditions in individuals is, of course, well proved by common observation on bodies of troops ordered into malarial regions. In such instances, it has been my experience that the vast majority of the infected men show clinical symptoms within from two to three weeks after entering the malarial district, and that the greatest number of clinical cases occur during the third week after exposure. Cases continue to develop, however, for several weeks, so that it must be admitted that the period of incubation varies greatly as between persons exposed to infection under the same conditions. It is probably safe to state that, under natural conditions, the usual period of incubation of malaria due to *Plasmodium vivax* is between fourteen and twenty-one days.

The period of incubation of malaria caused by *Plasmodium malariae,* the quartan plasmodium, after the direct inoculation of blood containing the parasite, was shown to be ten to fifteen days by Gualdi and Antolisei;[148] from eleven to eighteen days by di Mattei;[149] and twelve days by Baccelli.[140] More recently Kirschbaum[150] has shown that the incubation period of quartan malaria after the injection of blood containing *Plasmodium malariae* varies from four to ten days after intravenous injection and is about twenty-one days after subcutaneous injection.

The period of incubation of malaria due to *Plasmodium malariae* transmitted by the bites of infected mosquitoes under experimental conditions has averaged three weeks, but the natural period of incubation is still undetermined, owing to the rarity of this type of malaria.

147. James, S. P.: Pub. League of Nations, III; Health, III, 6.
148. Gualdi and Antolisei (footnote 8, first reference).
149. Di Mattei, E.: Riforma Med., no. 121, 544, 1891.
150. Kirschbaum, W.: Arch. f. Schiffs- u. Tropen- Hyg. **31**:349, 1927.

The period of incubation of malarial infection due to *Plasmodium falciparum* after direct inoculation of blood containing the plasmodium is shorter than in infections so produced by *Plasmodium vivax* or *Plasmodium malariae*. Bignami [151] found it to vary from six to ten days, and Bastianelli and Bignami,[151] from three to five days, the greater number of the experiments giving incubation periods between four and five days. As this species is not used in treating paretic patients, owing to the danger involved, there is no recent data regarding the incubation period after direct inoculation, but it is probably safe to say that it is usually between five and six days.

The period of incubation after the experimental production of malaria by the bites of mosquitoes infected with *Plasmodium falciparum* has been found by Bastianelli and Bignami [151] to be twelve days; by Schüffner,[152] fifteen days; and by Jansco,[153] to vary between ten and fourteen days, the average being about eleven days. Under natural conditions the period of incubation agrees roughly with that after the bite of mosquitoes in experimental infections. Marchiafava and Bignami [154] observed three instances of infection with this plasmodium in which the incubation period could be ascertained without question, and in one case the incubation period was nine days; in one, ten days and in the third, between nine and ten days. Jackson [155] described the occurrence of malaria in the members of a troop of United States Cavalry under circumstances in which the period of incubation could be accurately ascertained. Of forty-five soldiers belonging to this troop, exposed at the same time, under the same conditions, and removed at once to a nonmalarial region, no less than eighteen developed malaria due to *Plasmodium falciparum*, and the incubation period in all varied from ten to eleven days. From personal observation, I have noted that most of the infections with *Plasmodium falciparum* in soldiers are accompanied by symptoms within from ten to fourteen days after the first exposure in an infected region, and my experience is confirmed by that of many other observers who have studied this subject.

While the periods of incubation for the various plasmodia which have been mentioned are those usually observed, it should be remembered that often the period of incubation with all the malaria plasmodia is greatly prolonged, covering weeks or even months. I [21] described a case in which the period of incubation must have been at least seven months, and another in which the period of incubation was at least four months. In both of these cases the infection was with *Plasmodium*

151. Bastianelli and Bignami: Ann. d'Igiene **9**:272, 1899.
152. Schüffner, W.: Ztschr. f. Hyg. **61**:89, 1902.
153. Jancso, N.: Centralbl. f. Bakteriol. **36**:624, 1904.
154. Marchiafava and Bignami (footnote 20, first reference).
155. Jackson, T.: Am. Med. **8**:67, 1905.

*falciparum*. Recently Rombach [156] described an infection with *Plasmodium vivax* in which the incubation period was three months; de Jong,[157] a case, infected with the same species, in which the incubation period was about nine months; and Kollewijn,[158] two cases of infection with the same species having an incubation period of nine and twelve months, respectively. In these long periods of incubation it should be understood that plasmodia are sometimes present in the blood before the occurrence of symptoms, and that the only explanation of the absence of symptoms appears to be the natural resistance of the individual to the parasite. Ross and Thomson,[159] in 1910, showed that in order that symptoms be produced by *Plasmodium vivax*, for instance, there must be from 200 to 500 plasmodia per cubic millimeter of blood and by *Plasmodium falciparum*, from 600 to 1,500 per cubic millimeter of blood. Unless these figures are approximated, an infection with either plasmodium may remain symptomless indefinitely, and this is just what occurs in persons showing the long periods of incubation mentioned. They are able to keep the number of plasmodia below the "symptom number" for long periods of time, while in most persons no such resistance is encountered by the plasmodia and the incubation periods are the usual ones that have been described.

*Temperature Curves*.—Recent observations on induced malaria in patients with paresis, either through the bites of infected mosquitoes or by the direct inoculation of blood containing the plasmodia, has shown, at least in the instance of the tertian plasmodium (*Plasmodium vivax*), that the temperature curves are not as regular as generally believed, but that gross variations occur which are not at all characteristic of typical tertian infection. It has long been known that the temperature curve in malarial infections is of little real diagnostic value in differentiating between infections due to the various species of malaria plasmodia, except in typical cases, which are comparatively rare, and I [160] urged the fallacy of relying on the temperature curve in the diagnosis of malarial infections as long ago as 1901, although at that time temperature curves were supposed to be diagnostic of the various types of malaria. There is no question but that too much stress has been placed on the absolutely tertian character of the temperature curve in tertian infections and the quartan character in quartan infection, for so many factors influence the curve as mixed infections with different species of plasmodia, double or triple infections, the natural resistance of the patient, etc., that

156. Rombach, K. A.: Nederl. Tijdschr. v. Geneesk. **70**:1914, 1926.

157. De Jong, J. J.: Nederl. Tijdschr. v. Geneesk. **70**:163, 1926.

158. Kollewijn, J. R.: Nederl. Tijdschr. v. Geneesk. **70**:2084, 1926.

159. Ross and Thomson, quoted by Hehir; Malaria in India, New York, Oxford University Press, 1910, p. 216.

160. Craig, C. F. (footnote 21, first reference).

a diagnosis based on the temperature alone is often fallacious. The following quotation is taken from the article referred to: "Statistics of the type of malaria present in any locality, based entirely upon the character of the temperature curve are worse than useless and should receive but little consideration in the study of malarial endemicity." This statement has been confirmed by many investigators, and the recent experimental work in infections with *Plasmodium vivax* demonstrates that even in this common and supposedly typical infection a tertian temperature curve is often absent both at the beginning and end of the attack. Thus, in his experimental infections in paretic patients produced by infected mosquitoes, James [15] noted that the temperature curve after infection with *Plasmodium vivax* was often typically quotidian at the beginning of the attack, became tertian later, and in some cases the attack ended with a quotidian temperature curve. In other cases, the attack began with a tertian temperature which became quotidian later. St. John [143] had a similar experience in tertian infections produced in paretic patients by direct inoculation of blood containing *Plasmodium vivax*. He found that some of his patients ran a typically tertian temperature throughout the attack, but that in the majority, the temperature became quotidian. In some cases the attack began with quotidian paroxysms, the symptoms ceased for a few days, to be followed by typical tertian paroxysms. Similar observations have been recorded by practically all who have treated paretic patients by the induction of malaria, and there is no question that little dependence can be placed on the temperature curve in these cases, so far as the diagnosis of the type of infection is concerned. These variations, however, do not mean that at one time *Plasmodium vivax* segments in twenty-four hours and at another in forty-eight hours, but are explained by the fact that certain of the plasmodia tend to "lag" in their development and do not segment at regular intervals, so that not all of them segment at approximately forty-eight hours, but enough are segmenting every day to produce symptoms, although the individual plasmodia are not segmenting every twenty-four hours. The condition is really a double infection with the two generations segmenting on alternate days, thus producing a quotidian temperature curve. In some of these patients, at the beginning of the attack there is an irregular continuous curve which is succeeded by a typical tertian temperature.

In considering these variations from the classical tertian temperature curve shown in patients with paresis who have been infected with malaria, it should be remembered that persons are being considered in whom pathologic changes have occurred in the nervous system and it may be that their reaction to the infection differs markedly from the reaction of the normal, healthy person. Certain it is, that the majority of natural infections with *Plasmodium vivax* that are seen by the physi-

cian show a typical tertian temperature curve with tertian paroxysms, and one should be careful in drawing sweeping conclusions regarding malarial infection under natural conditions from the results obtained in treating patients with paresis by the induction of malaria experimentally.

*General Symptomatology.*—The protean character of the symptoms of malarial infection has always been recognized, and recent observations merely serve to accentuate this fact. Like syphilis, malaria may affect almost every portion of the human body and the symptoms present will vary with the portion affected. Some of the most important contributions to the knowledge of the symptomatology of malaria will be briefly summarized.

*Respiratory System.*—The subject of a lobar pneumonia due to the malaria plasmodia has always attracted attention and numerous cases are on record in the literature. I believe that the evidence is not sufficient to prove that a true lobar or lobular pneumonia can be caused by the malaria plasmodia, but there is no question that symptoms are observed which indicate a localization of the plasmodia in the lung and which closely simulate lobar or lobular pneumonia. In a recent study of this question, Karve [161] states that there occurs a primary malarial pneumonia characterized by slight dulness, enlarged spleen, diminution of breath sounds, a slightly blood-stained sputum, an increased pulse and respiration rate, and the fact that it is cured by the administration of quinine. I [133] have described a form of pernicious malaria in which the symptoms are similar to those of lobar pneumonia although the temperature curve is intermittent and the symptoms are improved or disappear during the intermissions. I have also observed cases in which the symptoms were indistinguishable from those of lobar pneumonia and the temperature curve similar to that of the latter infection. In such cases, the symptoms are undoubtedly due to localization of the plasmodia in the lungs, just as the cerebral symptoms of malaria are due to localization in the brain. Several cases of malarial infection in which the symptoms included those of asthma have been reported in the literature, and Auday and Arana [162] have recently described an interesting case of this kind in which the administration of quinine gave almost instant relief.

*Circulatory System.*—During malarial attacks there is frequently a functional systolic murmur heard over the mitral valve, most pronounced in long continued infections, due to the anemia present, and there may be some shortness of breath during the malarial attack, while the slow pulse of the malarial convalescent is well known. In a recent

161. Karve, S. D.: Kenya M. J. **3**:116, 1926.

162. Auday and Arana: Semana méd. **33**:1214, 19.

review on the heart in malaria, Benhamou [163] calls attention to murmurs, low arterial tension and the slow pulse, but stresses a lesion which he has observed and considers typical of malarial infection. It consists in a spherical dilatation of the heart producing back pressure, edema, ascites, fluid in the pleura and an enlarged liver. Treatment with quinine and digitalis usually results in complete recovery. In a large experience, I do not recall observing such cases, but this may be due to the fact that early treatment with quinine prevented their development.

*Gastro-Intestinal System.*—The symptoms of malarial infection which originate in the gastro-intestinal system are usually nausea and vomiting during the paroxysm, anorexia, and constipation or diarrhea. However, there may be a localization of the malaria plasmodia in the intestinal tract and certain forms of pernicious malaria described as cardialgic, choleraic or dysenteric may result. Recent literature contains many references to cases in which the symptoms originated in the stomach or intestines. Thus, Jhaveri [164] describes five cases of malarial infection in which severe abdominal pain and tenderness, constipation, vomiting, high temperature and collapse occurred, and in which treatment by intramuscular injection of quinine resulted in recovery. Bonorino, Udaondo and Sanguinetti [165] describe numerous cases of malarial gastritis and enteritis and note that the acidity of the gastric juice may either be increased or decreased during a malarial attack, Strachan [166] described a case of malaria in which there was severe diarrhea, there being as many as twenty-five stools daily, with blood and mucus, followed by death. Autopsy did not show any ulceration, but there were scattered areas of submucous hemorrhage. Similar observations have been recorded by Falconer and Anderson [167] in a series of cases of malarial dysentery in which ulcerations were not present but in which numerous submucous hemorrhages occurred. Karve [168] has recently reviewed the disorders of the digestive system due to malaria, and considers them under the headings stomatitis, gastritis, diarrhea and dysentery. Malarial stomatitis is aphthous, offensive, very painful and occurs in children and clears up under the administration of quinine; gastritis, diarrhea and dysentery usually occur during the fall in temperature and the most effective treatment is quinine and emetine.

There are numerous references in the literature to malarial appendicitis and it is true that severe abdominal pain in the region of the

163. Benhamou, E.: Paris méd. **16**:25, 1926.

164. Jhaveri, M. H.: Indian M. Gaz. **61**:26, 1926.

165. Bonorino; Udaondo and Sanguinetti: Arch. Arg. del Ap. Digestivo. **1**: 817, 1926.

166. Strachan, A. S.: M. J. South Africa **22**:13, 1926.

167. Falconer and Anderson: Trop. Dis. Bull. **12**:44, 1915.

168. Karve, S. D.: Kenya & E. Africa M. J. **4**:95, 1927.

appendix is sometimes present during malarial paroxysms and may lead to a diagnosis of appendicitis. I have observed several cases in which this diagnosis was made and operation was advised, and in which the condition cleared up under the administration of quinine. Recently, Bressot [169] has described five cases of malaria simulating appendicitis, in which there was acute abdominal pain, bilious vomiting and abdominal tenderness, especially over the region of the appendix. Two of the patients refused operation and recovered under the administration of quinine. In the other three cases the abdomen was opened and the appendix found to be normal, but there was a striking hyperemia of the intestinal wall affecting the submucous coat of the lower end of the ileum and the large intestine. In two of these cases there were also ecchymotic, edematous, infiltrated plaques on the mucous membrane. Four of these cases showed infection with *Plasmodium vivax* and one with *Plasmodium falciparum*.

The question as to whether the malaria plasmodia can actually produce lesions in the appendix is still open. Some authorities believe that the localization of the plasmodia in the capillaries of the organ, together with the stoppage of the vessels by pigment and parasites, can produce necrosis and the lesions of appendicitis, while others believe that there is no actual lesion in the appendix but that the symptoms of the malarial infection simply resemble those of appendicitis. Thus, Demjanow [170] claims that the appendix reacts to infection with the plasmodia as to other infections, by hypertrophy of its lymphatic system, and that the blocking of the capillaries of the organ by thrombi formed of pigment may cause appendicitis. On the other hand, Basile [171] claims that it is not correct to speak of malarial infection as simulating appendicitis. I can see no reason why the localization of large numbers of plasmodia and of pigment in the capillaries of the appendix might not give rise to congestion, inflammation and necrosis, with the consequent production of the symptoms of an acute or subacute appendicitis.

*Nervous System.*—The usual symptoms connected with the nervous system in malaria are well known, but in the recent literature on the subject there are recorded many instances of unusual nervous symptoms accompanying the various types of malarial infection. Amosov and Lindtrop [172] describe several cases of malaria in which ataxia was a prominent symptom, all with malignant tertian infections (*P. falciparum*). In three of these cases there was an acute period of three days' duration with active symptoms of malaria and acute ataxia; the

169. Bressot, E.: Presse méd. **35**:707, 1927.
170. Demjanow: Arch. f. Schiffs- u. Tropen- Hyg. **31**:89, 1927.
171. Basile, G.: Policlinico **34**:1143, 1927.
172. Amosov, M., and Lindtrop: Russ. J. Trop. Med. **4**:3, 1925.

second period, lasting about a week, was associated with improvement in the symptoms; while the third period is described as the period of latency. During this period, mental depression and incoordination disappeared and recovery ensued. All of the patients were successfully treated with quinine. Trabaud [173] describes a case of peripheral neuritis affecting the sciatic nerve in which the blood was positive for *Plasmodium vivax* and in which the patient recovered under treatment with quinine. De Vries [174] describes a case characterized by cerebellar ataxy, increased reflexes, intention tremor, slow and slurring speech and nonconvergence of the eyes, caused by infection with *Plasmodium falciparum*. Urchs,[175] in a discussion of the relation of malaria to the nervous system, states that the cases observed by him fall into three classes: those accompanied by damage to the peripheral nerves, including trigeminal neuralgias; herpes zoster, and cases showing symptoms of encephalitis and leptomeningitis.

As regards meningeal symptoms, it has long been known that certain cases of pernicious malaria of the cerebral type are accompanied by symptoms of meningeal involvement, and some authorities describe a meningeal type of pernicious malaria. While this classification may be hardly justifiable, it is a fact that malarial infections are rarely observed in which the symptoms are almost indistinguishable from those of cerebrospinal meningitis. A case in point is that reported by Teixeira [176] in which Kernig's sign, dilated pupils and contraction of the muscles of the neck and lumbar region were present, while there was indifference to his surroundings, disconnected speech and lack of comprehension and irresponsiveness when addressed. This patient was diagnosed as suffering from epidemic cerebrospinal meningitis, and it was not until the blood was examined and *Plasmodium vivax* was found that the true condition was appreciated. Treatment with quinine resulted in prompt recovery.

*Masked Malaria.*—The term, masked malaria, is applied to malarial infections in which the symptoms are masked by those of a complicating disease, or in which the symptoms so closely resemble those of some other disease that a differential diagnosis is impossible without the demonstration of the malaria plasmodia in the patient's blood. In 1903, I [177] called attention to the frequency of masked malaria and stated that of 1,267 cases of malaria that I had observed in which the plasmodia were demonstrated in the blood, no less than 395, or about 32 per cent,

173. Trabaud, C.: Rev. Med. et Hyg. Trop. **18**:33, 1926.
174. De Vries, E.: China M. J. **41**:503, 1927.
175. Urchs, O: Arch. f. Schiffs- u. Tropen- Hyg. **31**:365, 1927.
176. Teixeira, E.: Rev. med.-cirrurg. do Brazil **34**:292, 1926.
177. Craig, C. F.: Am. Med. **6**:145, 1903.

were either latent or masked infections. Of these, 118 were masked infections and were about equally divided between those in which a complicating disease masked the malarial infection and those in which the symptoms of malaria resembled those of some other disease process. In 1928, I [178] again called attention to this subject and published the following tables illustrating the two types of masked malarial infection. Table 2 shows the clinical diagnosis in ninety-one cases of masked malaria in which the symptoms of the disease diagnosed masked those due to malaria and the malarial complication was discovered through a routine examination of the blood.

As already stated, the symptoms of malaria frequently closely simulate those of other infections and diseases, and among the most common of these atypical symptoms, which often cause difficulty in the diagnosis of the malarial infection, may be mentioned various

TABLE 2.—*Cases of Masked Malaria Complicating Other Diseases*

| Disease | Number of Masked Cases of Malaria |
|---|---|
| Chronic dysentery | 45 |
| Chronic diarrhea | 5 |
| Pulmonary tuberculosis | 18 |
| Amebic dysentery | 4 |
| Acute bronchitis | 2 |
| Measles | 3 |
| Typhoid fever | 2 |
| Pneumonia | 2 |
| Gonorrhea | 4 |
| Rheumatism, articular | 1 |
| Abscess of liver | 1 |
| Furunculosis | 1 |
| Diabetes mellitus | 1 |
| Cellulitis | 1 |
| Appendicitis | 1 |
| | 91 |

periodical neuralgias, especially of the fifth nerve, the intercostal nerves and the occipital region; convulsions, especially common in malarial paroxysm in children; various nervous symptoms such as insomnia, delirium, coma, mania or melancholia; while some of the infectious diseases as typhoid, pneumonia, yellow fever, pulmonary tuberculosis and cerebrospinal meningitis may be closely simulated by the symptoms of malarial infection.

Table 3, in which the diagnosis of the disease mentioned was made before the malarial nature of the condition was discovered by the finding of the malaria plasmodia in the blood, well illustrates how malaria simulates the symptoms of other disease processes, and how valuable a microscopic examination of the blood is in the diagnosis of disease.

178. Craig, C. F.: Oxford Medicine, New York, Oxford University Press, vol. 5, p. 781.

In all of these cases, the malarial infection cleared up under prompt and proper treatment with quinine and the symptoms leading to the erroneous diagnosis disappeared.

*Congenital Malaria.*—The possibility of the transference of the malaria plasmodia from the mother to the fetus has always been a moot question in the pathology of malaria. In the older descriptions of malarial infections, before the discovery of the plasmodia, it was generally accepted that this occurred and the large infant mortality rate in malarial regions was accredited to prenatal infection. The discovery of the malaria plasmodia, and the method of natural transmission of the fevers produced by these parasites, rendered most of the evidence of the older writers in this respect practically worthless, as none of the cases reported had been examined as to the presence of the plasmodia in the blood or tissues, and the large infant mortality could be easily accounted for by infection after birth, as it was soon demonstrated that the transmitting mosquitoes bit the youngest infants when exposed to their bites.

TABLE 3.—*Erroneous Diagnosis in Cases of Masked Malaria*

| Diagnosis | Number of Cases |
|---|---|
| Anemia | 20 |
| Chronic dysentery | 15 |
| Typhoid fever | 10 |
| Chronic diarrhea | 6 |
| Pulmonary tuberculosis | 5 |
| Acute bronchitis | 4 |
| Insolation | 6 |
| Melancholia | 4 |
| Malta fever | 6 |
| Rheumatism, muscular | 4 |
| Neuralgia, facial | 3 |
| Pneumonia, lobar | 3 |
| Appendicitis, acute | 2 |
| Syphilis | 2 |
| Insanity | 2 |
| Peritonitis | 1 |
| Jaundice, acute catarrhal | 1 |
| Cerebrospinal meningitis | 1 |
| Yellow fever | 1 |
| | 96 |

After the discovery of the malaria plasmodia, many authorities made examinations of newly born infants, or of the fetus in utero in women who had died from pernicious malaria, and until comparatively recent dates evidence was not obtained that the parasites were ever transferred from the mother to the fetus. Thus, Bignami [179] examined the blood of a fetus of three months' gestation and another of six months', from women who had aborted because of pernicious attacks of malaria, with a negative result in both instances; in a third case, that of a pregnant women who died of pernicious malarial fever, the autopsy showed the usual lesions found in such cases, and the blood contained multitudes of plasmodia, but the blood of the fetus was free from

179. Bignami: Suppl. al Policlin. **4**:763, 1897.

plasmodia and the organs did not show any malarial lesions. Sereni,[180] in an elaborate study of this subject, concluded that placental transmission was impossible, as he had never observed the parasites in newly-born infants of mothers suffering at the time of birth from malarial infection, although he stated that the placenta contained numerous plasmodia. Ziemann,[181] in the Kameruns, had never observed plasmodia in the blood of newly-born negro infants, although in four cases the mothers were suffering from malaria at the time of birth. He believed that the placenta offered an impassable barrier to the plasmodia. Thayer[182] details an interesting and conclusive case, that of a woman suffering from a severe quartan infection, who gave birth to a seven months' fetus during a paroxysm of the fever. The blood of this child did not show any plasmodia nor did a malarial infection develop later, but an examination of the placenta developed the interesting and significant fact that while on the maternal side numerous plasmodia could be demonstrated, there was a complete absence of them on the fetal side of the organ. I have also observed this distribution of the plasmodia in the placenta in cases of malarial infection in women who have given birth to children during an attack of malaria.

While the observations mentioned appear to prove conclusively that congenital malaria is impossible, it must be admitted that today just as conclusive evidence is possessed that the transference of the malaria plasmodia to the fetus in utero is possible and does occur, although very infrequently. There are a few instances of congenital malaria in the recent literature that are proved adequately from a scientific standpoint, so that it must be admitted that at times the malaria plasmodia pass from the mother to the fetus through the placenta and that this organ does not always present an impassible barrier to such passage. Among the somewhat numerous instances of congenital malarial infection reported in the recent literature, the following are some of those that are undoubted instances of such infection.

In 1925, Buckingham[183] reported two cases of congenital malaria. In one case, the mother was suffering from malaria at the time of the birth of the child, and the child's blood was examined and found positive for malaria plasmodia before the placenta was expelled. In the other case, the mother, a Chinese woman, was suffering from malaria while in labor. The presentation was a transverse one and the hand of the fetus prolapsed. This hand was cleansed and blood films made, after which version was performed, and the child extracted.

180. Sereni, S.: Bull. d. Acad. di med. di Roma, 1903, part 1-2-3.

181. Ziemann, H.: Handbuch der Tropenkrankheiten, Mense, Hamburg, 1906.

182. Thayer, W. S.: Malaria, in Allbut: System of Medicine, Tropical Diseases, New York, The Macmillan Company, 1907.

183. Buckingham, E. W.: China M. J. **34**:1140, 1925.

The examination of the blood films was positive for *Plasmodium malariae*. There can be no doubt that in these instances the infants were infected with malaria plasmodia before birth. Weselko [184] states that in fourteen cases he found malaria plasmodia in the peripheral blood of mother and child immediately after the birth of the child, and that in four cases the plasmodia were found in the blood of the cord. Five of these children died from the malarial infection within forty-eight hours after birth. Annecchino [185] describes the case of a child born in a nonmalarious region of a malarious mother who had been untreated for fear of quinine producing an abortion. Forty-eight hours after birth the child had convulsions and the spleen and liver were found enlarged, the mother having a malarial paroxysm at the same time. The condition recurred in forty-eight hours and at this time the blood of the child showed schizonts and gametocytes of *Plasmodium vivax*. Dorolle and Dang-Huu-Chi [186] record the case of a woman with a five months' pregnancy, who died from pernicious malaria, and who, at autopsy, showed retroplacental hemorrhagic foci with infarcts. The blood from the cord showed schizonts and segmenting forms of *Plasmodium falciparum* and considerable pigment. The authors believe that in this case the congenital infection was due to the rupture of the blood vessels in the placenta which produced the hemorrhages and allowed the passage of the plasmodia into the cord. In view of the observations of many authorities regarding the location of the malaria plasmodia in the capillaries of the maternal side of the placenta and their absence in the vessels of the fetal side, it is probable that congenital infection cannot occur unless there is some lesion in the placenta allowing the parasites to enter the fetal side of the organ.

While congenital malaria is rare, it is a fact that malarial infection in the mother has a profound effect on the child. A most interesting and valuable contribution to this subject has been recently published by Blacklock and Gordon.[187] They examined 173 pregnant women at Freetown, Sierra Leone, only nine of whom showed *Plasmodium falciparum* in the peripheral blood, but of 155 placentas of the 173 women, no less than 59 showed *Plasmodium falciparum*, i.e., 38 per cent. These organs were examined carefully in sections and although numerous plasmodia were present on the maternal side of the placentas, in no instance were parasites found on the fetal side of the organs, in the umbilical cord or in the circulation of the infants. However, it was found that of fifty-one children born of mothers with an infected placenta, 25.5 per cent died within seven days, while of ninety-three

184. Weselko, O.: Arch. f. Schiffs- u. Tropen- Hyg. **30**:73, 1926.
185. Annecchino, F. P.: Riforma Med. **42**:285, 1926.
186. Dorolle and Dang-Huu-Chi: Bull. Soc. path. Exot. **20**:589, 1927.
187. Blacklock and Gordon: Ann. Trop. Med. **19**:327, 1925.

children born of mothers with an uninfected placenta, only 5.4 per cent died within that time. The authors suggest that while there was no evidence of the transmission of the malaria plasmodia from mother to child in these cases, the early death of so many of the children may be due to the effect of a toxin liberated at the time of segmentation of the plasmodia in the maternal side of the placenta.

### DIAGNOSIS

Although many attempts have been made to devise other methods of diagnosis, the finding of the malaria plasmodia in the blood of the infected person is still the only reliable method of distinguishing malarial infections from other infections and diseases. The symptoms of malaria are so protean and resemble those of other disease processes so often that a diagnosis based on symptoms alone is worthless except in absolutely typical infections with the tertian and quartan plasmodia, and the comparatively rare infections with *Plasmodium falciparum* in which a typical temperature curve is exhibited. Fortunately, the plasmodia can usually be demonstrated with little difficulty in stained blood preparations in the vast majority of malarial infections. As I have stated elsewhere:[188]

> It has not been my experience, which has covered over thirty years and the examination of many thousands of blood smears from malarial patients, that cases of infection occur in which the plasmodia are so few in number that it is impossible to demonstrate them by an examination of stained blood smears, if symptoms of the infection are present, and I have yet to see a case of malaria severe enough to give rise to even slight symptoms, in which it was impossible to demonstrate the plasmodia in the peripheral blood, if repeated examinations were made, provided quinine had not been administered. On the other hand, I have observed malaria plasmodia in hundreds of cases of latent malarial infection in which no symptoms were present at the time of examination.

In order to increase the chances of finding the plasmodia in the peripheral blood in latent cases and in cases presenting clinical symptoms but in which few parasites are present, the various so-called "thick-film" methods have been evolved, all depending on the fact that by their use a larger amount of blood is concentrated in a given space and thus the number of plasmodia increased through such concentration. These methods have proved of great use in making malarial surveys and in the diagnosis of suspected latent malaria, but I have never found the use of a thick-film blood preparation necessary in the diagnosis of clinical malaria. There have been many modifications of the original Ross "thick-film method" published in the literature, and almost every investigator has described a method of his own which he regards as superior to others in use, but none of them have succeeded in obviating the

188. Craig, C. F. (footnote 21, seventh reference).

greatest obstacle to the use of the thick-film in practice, i. e., the distortion of the plasmodia so that it requires considerable practice to recognize them and to differentiate the exact species present. While the thick-film methods do greatly increase the chances of finding the plasmodia in latent malarial infections, it is true that they are not necessary in the diagnosis of clinical malaria and will never replace the examination of the thin film in ordinary work in the clinical laboratory. The true field of the "thick-film" in examination of the blood is in malarial surveys where it is of the utmost service in the hands of experts in determining the endemicity of malaria.

Measures for increasing the number of plasmodia in the peripheral blood in suspected infections have been devised and some of them are useful.

The application of the x-ray or of ice to the abdomen over the region of the spleen has been found to increase the number of malaria plasmodia in the peripheral blood by some observers, but the most successful method of accomplishing this purpose is the administration of certain drugs, which, by contracting the blood vessels of the viscera, as the spleen, force the plasmodia into the peripheral circulation. Of the drugs that have been used for this purpose an aqueous extract of ergot, strychnine, and epinephrine, administered hypodermically, have given the best results, especially the latter. Sarsen,[189] in an excellent review of all of the provocative methods of diagnosis in malaria, concluded that the subcutaneous administration of 1 mg. of epinephrine, and the use of ultraviolet ray over the splenic area, with physical exercise, were the best methods. Dazzi [190] found that the subcutaneous administration of 1 mg. of epinephrine was by far the best method, in his experience, for forcing the plasmodia into the peripheral blood. He found that the parasites began to appear in the peripheral circulation about twenty minutes after the administration of the drug, and that the maximum number was reached about an hour after the injection. In his experience, the plasmodia disappeared from the blood about twenty-four hours from the time of the administration of the epinephrine. On the other hand, Di Pace [191] secured the best results by the subcutaneous injection of from 2 to 3 mg. of strychnine nitrate, plasmodia being found in the blood in many doubtful cases after the administration of this drug.

It is rarely that one has to use provocative methods in the diagnosis of the plasmodia, for usually the parasites are present in the blood in sufficient numbers to be demonstrated in stained films of the peripheral blood if clinical symptoms are present. If the symptoms are suspicious

189. Sarsen, P.: Arch. f. Schiffs- u. Tropen- Hyg. **23**:235, 1919.
190. Dazzi, A.: Policlinico **26**:1413, 1919.
191. Di Pace, E.: Trop. Dis. Bull. **20**:295, 1923.

and repeated examinations of blood films are negative, the subcutaneous administration of epinephrine will sometimes clear up the diagnosis.

*Splenic Index.*—Enlargement of the spleen is always present at some time during malarial infection and this fact has been taken advantage of in the diagnosis of doubtful clinical cases and of latent infection. The number of enlarged spleens encountered in the examination of the inhabitants of a locality is known as the "splenic index" and is considered indicative of the amount of malaria present in that locality. In the discussion of the prophylaxis against malaria, this subject will be considered more fully, for it is in malarial surveys of localities that the splenic index is most useful, but in the diagnosis of clinical malaria it is not of much service, as the spleen is enlarged in so many other acute infections that, of itself, this symptom is of little value, and mere enlargement of this organ in a fever case should not be considered as diagnostic of the malarial nature of the condition. If the splenic enlargement is due to malaria and fever is present, an examination of the blood will almost invariably result in the demonstration of the presence of the plasmodia, and if repeated examination fail to demonstrate the plasmodia some other cause for the enlargement of the spleen should be considered.

*Serologic Methods of Diagnosis.*—Within recent years efforts have been made to devise some serologic method of diagnosis for malarial infections, and both complement fixation and precipitation tests have been described and have apparently given good results in the hands of their originators. At the present time none of these tests has been placed on a practical basis, but it is not too much to hope that continued research will develop an accurate and practical serologic method for the diagnosis of malarial infection.

Thomson and Thomson [192] were the first to attempt to prepare an antigen for use in a complement-fixation test in the diagnosis of malaria. This antigen was prepared by extracting cultures of the malaria plasmodia grown in dextrose-blood, and complement fixation occurred in some cases of infection in which this antigen was employed, but the antigen was so anticomplementary that it could not be used in practical diagnostic work. Later, J. G. Thomson,[193] using an antigen prepared by extracting the blood of patients rich in plasmodia, secured complement fixation in numerous instances, but found that the reaction was not specific for the various species of plasmodia, as antigens prepared by extracting the blood of patients infected with *Plasmodium falciparum* gave positive reactions with the blood serum of patients infected with *Plasmodium vivax* and vice versa. Horowitz and Wlassowa,[125] using

192. Thomson and Thomson: Proc. Roy. Soc. **87**:77, 1913.

193. Thomson, J. G.: Brit. M. J. **2**:628, 1918.

antigens prepared by extracting the placentas of women infected with malaria, or extracts of red blood corpuscles of malarial patients, obtained complement fixation in certain cases and concluded that a complement-fixing antibody is produced during a malarial infection which can be demonstrated within two weeks after infection and as long as five years after such infection.

In 1927, Manson-Bahr [194] approached the problem from a different angle. He attempted to secure a reliable antigen by extracting with absolute alcohol the stomachs of anopheline mosquitoes infected with the human malaria plasmodia and showing numerous oocysts. By reason of experimental difficulties, he was unable to secure enough of such material for an extract and he transferred his efforts to malaria in birds and secured an extract of twenty-four proteosoma infected stomachs of mosquitoes with which he tested the blood serum of several patients suffering from infections with *Plasmodium falciparum* and *Plasmodium vivax*, but with negative results in all cases. Still more recently, Kingsbury [195] has studied the subject extensively, and has tested eleven antigens, eight made from the spleen, liver, heart, brain and blood of a fatal case of infection with *Plasmodium falciparum*, and three from the blood of a patient showing immense numbers of *Plasmodium vivax*. He found that the most satisfactory antigens were saline emulsions of washed and lysed blood. With such antigens he tested twenty-five cases of infection with *Plasmodium falciparum* and 48 per cent gave a positive result with the *Plasmodium falciparum* antigen, while sixteen tested with *Plasmodium vivax* antigen resulted in 31 per cent of positive results with the blood serum of persons infected with other parasites, the *Plasmodium falciparum* antigen, 50 per cent gave a positive result and of six cases tested with the *Plasmodium vivax* antigen, 67 per cent were positive. It will thus be seen that the reaction was not specific for the two species of plasmodia used in the experiments although a higher percentage of positive results were obtained with homologous antigens.

Recently, endeavors have been made to evolve a precipitin test for the diagnosis of malarial infection. In 1927, Taliaferro, Taliaferro and Fisher [127] described such a test, using as antigens extracts, in Coca's fluid, of heavily infected peripheral blood or placentas of women infected with malaria. With such antigens they obtained positive precipitin reactions with the blood serum from malarial patients and negative results with the blood serum of persons infected with other parasites, such as amebae, flagellates, worms, etc. They regarded the reaction as a specific antigen-antibody reaction which might prove of diagnostic value. They obtained positive results with the blood serum of forty-five

194. Manson-Bahr, P.: Tr. Roy. Soc. Trop. Med. & Hyg. **21**:63, 1927.

195. Kingsbury, A. N.: Tr. Roy. Soc. Trop. Med. & Hyg. **20**:359, 1927.

of fifty-four persons whose blood showed the malaria plasmodia, and of thirty-two persons having a negative thick blood film, six gave a positive precipitin reaction. In a later contribution, Taliaferro and Taliaferro [128] give the results of further observations on the precipitin reaction in malaria. They have found the most efficient antigens were extracts with ether and Coca's fluid of minced infected placentas, the antigen used being the aqueous portion obtained by extracting the ether insoluble portion with Coca's fluid. The $p_H$ must be 7.8, as acid antigens were found to be inactive. Of thirty-two serums from persons infected with malaria, four gave a four plus reaction, eleven a double plus reaction, and fifteen a single plus reaction. Of thirty-two persons free from malarial infection, so far as could be ascertained, one gave a single plus reaction, one was doubtful, and twenty-seven were negative.

The results of the foregoing precipitin test appear to be significant and indicate that much may be expected of research devoted to this subject, but it cannot be said that at present either complement fixation or precipitin tests are of real practical value in the diagnosis of malarial infection.

Much has appeared in the literature regarding the value of a diagnostic method for malarial infection based on the demonstration of urobilin in the urine. Kervrann [196] recommends Schlesinger's solution for this purpose, which consists of 10 per cent of zinc acetate in alcohol. This is thoroughly shaken and mixed with an equal amount of the suspected urine, a few drops of weak tincture of iodine added, and the mixture filtered. If urobilin is present the filtrate is fluorescent, and some authorities regard this reaction as diagnostic of malarial infection. Kervrann found the reaction positive in 72 per cent of cases of malaria in which the blood showed plasmodia. Infections with *Plasmodium falciparum* gave 70 per cent positive results, with *Plasmodium vivax*, 83 per cent positive results, and with *Plasmodium malariae*, 75 per cent positive results. He found that positive results were also obtained in pneumonia and abscess formation, so that the reaction is not a specific one. Kervrann's results have been similar to others and it is not considered that the reaction is specific for malaria, although it is suggestive, and might be of some service in differential diagnosis in selected cases.

## PROPHYLAXIS

At no time in the history of medicine has the prevention of the malarial infections attracted as much attention from the medical profession and public health officers as at present. The vast importance of these infections to the various armies engaged in the World War in certain regions, and the great epidemics of malaria that have swept across certain countries, as Russia, following that war, have anew

196. Kervrann, A.: Ann. de méd. et de pharm. colon. **23**:340, 1925.

focused attention on malaria and stimulated the interest in prophylaxis against the disease. While no discoveries have been made that affect the fundamental principles on which the prophylaxis against malaria are based, a great deal has been added to the knowledge of the subject within recent years as the result of data gained in the practical application of the various methods that have been recommended and of laboratory studies regarding the malarial plasmodia and the transmitting mosquitoes. More and more has it become evident that no one method of prophylaxis should be used to the exclusion of others, except under exceptional circumstances, but that every method available should be employed if one desires to secure the best results in the prevention of these infections. Several years ago, I [197] urged this principle in prophylaxis against malaria, although at that time the destruction of mosquitoes was generally held to be the only really valuable method of prophylaxis, and its truth has recently been sustained in the report of the League of Nations, Health Organization [198] in which it is stated that of the measures employed for the prevention of malaria there is no one that can be said to be the method of unquestioned choice, but that local conditions must decide the method or methods of prophylaxis to be adopted.

All prophylactic methods are based on three general principles: (1) the destruction of the mosquitoes transmitting the malaria plasmodia; (2) the protection of man from the bites of the transmitting mosquitoes, and (3) the destruction of the malaria plasmodia while in the human hosts. If conditions were ideal the application of any one of these principles would result in the disappearance of malaria in any region, but practically it is usually necessary to employ methods based on all three of these principles if success is to be obtained.

*Malarial Surveys.*—Before attempting the prophylaxis against malaria, a careful malarial survey should be made of the region, for unless this is done the information on which prophylaxis must be based will be lacking and success cannot be expected. Many models for malarial surveys have been published from time to time and any one who is interested will find excellent descriptions of the methods of conducting such surveys in the works of Ross,[9] Craig,[197] Parsons,[199] Hehir [200] and Knowles and Senior-White.[201]

197. Craig, C. F.: Bull. no. 6, War Dept., Surg. General's Off., Govt. Print. Off., Washington, D. C., 1914.

198. Report League of Nations, Health Organization, Second Report of the Malarial Commission, Geneva, 1927.

199. Parsons, A. C., in Byam and Archibald: Practice of Medicine in the Tropics, New York, Oxford University Press, vol. 2, p. 1554.

200. Hehir, P.: Malaria in India, New York, Oxford University Press, 1927.

201. Knowles and Senior-White: Malaria: Its Investigation and Control, Calcutta, Thacker, Spink & Co., 1927.

Such a survey should cover the available health statistics of the region as to the morbidity and mortality of malaria; the breeding places of anopheline mosquitoes; the species of anopheline mosquitoes present; the examination of the peripheral blood of as many children and adults as possible, especially of children between the ages of 1 and 10 years, in order to determine the "parasite index"; and the palpation of the spleens of children and adults for enlargement, in order to determine the "splenic index." The data accumulated should be carefully considered and the terrain of the locality studied, in order to ascertain what methods of prophylaxis will be most successful and most economical. Some authorities appear to be satisfied with a mosquito and spleen survey, regarding the presence of a high "splenic index" as conclusive evidence of the presence of much malarial infection, but this is not always true, especially in regions in which other infections are endemic which are accompanied by splenic enlargement or among certain natives in endemic malarial regions, nor is lack of enlargement of the spleen always indicative of the absence of malarial infection in a given locality. Thus, Clark,[202] in a valuable study of the relative merits of the parasitic index and the splenic index in the diagnosis of malarial infections in Haiti, has demonstrated that in adult Haitian negroes there is little enlargement of the spleen in persons infected with malaria, and that the splenic index is low in these natives while the parasitic rate is high, and this is true, to a less extent, of the children. Employing the thick blood film on 11,000 adult Haitian negroes, the parasite rate was found to be 23.5 per cent, while the same test on 1,102 of their children showed a parasite rate of 41.9 per cent. On the other hand, the splenic rate of the same adults was only 3.05 per cent and of the same children only 22.78 per cent. Of the 2,585 adult negroes who were found positive for the malaria plasmodia, only 110 had palpable spleens and of the 464 children whose blood contained the plasmodia, 175 had palpable spleens. It follows that 2,475 adults and 287 children with malaria would have been overlooked had the splenic index alone been considered as diagnostic of infection, and thus an erroneous idea would have been gained regarding the prevalence of malaria in the localities in which these negroes lived. The observations of Clark demonstrate that in malarial surveys the "parasite index" should always be obtained, whatever may be done regarding the "splenic index."

The data obtained during the malarial survey should be entered on a large map of the region surveyed, and when this is completed the whole picture of the prevalence of malaria in the district, the reasons for the prevalence, and the best methods for combating the infection, should be apparent to the well trained epidemiologist.

202. Clark, H. C.: Am. J. Trop. Med., to be published.

*The Destruction of Mosquitoes in Prophylaxis.*—The destruction of the mosquitoes is the ideal method of prophylaxis against malaria when conditions are such as to make it applicable and the cost is not too great. A survey to determine the exact species of anopheline mosquitoes present in the locality is most essential for it has been shown by numerous observers that certain species are more important transmitters of the malaria plasmodia than are others and that no attention need be paid to the destruction of the species seldom concerned in transmission, thus enabling one to accomplish the destruction of the really important transmitters at a less cost than would attend efforts to destroy all anopheline mosquitoes in the locality. The subject of the important mosquito transmitters of malaria has already been considered in discussing the etiology and epidemiology of malaria, and it is the consensus of opinion of all field-workers in prophylaxis against malaria that the most efficient and economic method of attacking the mosquito problem is to first ascertain the species of mosquitoes breeding in the region and then to devote time and money only to the destruction of the species that are known to be efficient transmitters of the plasmodia.

The destruction of mosquitoes may be accomplished at any portion of their life history, and the methods available will not be considered here as they are well known and are fully described in every recent work on malarial infections. Certain advances in knowledge regarding the transmitting insects and methods of destroying them may be briefly mentioned because of their practical importance and the fact that only the most recent works on malaria mention them.

The length of flight of the mosquitoes transmitting malaria has been grossly underestimated in the past, for it is now well proved that these insects will take long flights in order to reach human beings. In 1906, I [203] stated that anopheline mosquitoes would fly over 2 miles in order to secure a feeding of blood. This statement, made as the result of personal observation, was received with incredulity by entomologists and others, but it has since been abundantly confirmed by observations in the Canal Zone and in American camps during the World War, as well as elsewhere. This point is of practical importance in the prophylaxis of malaria with reference to the area to be covered in antimosquito operations. Darling [204] and others in the Canal Zone, and Bascom,[205] at Ebert Field, Arkansas, have proved that anopheline mosquitoes frequently fly as far as 2 miles in search of food, and Wenyon [95] states that they may fly as far as 3 miles. Bascom's

203. Craig, C. F. (footnote 59, first reference).

204. Darling, S. T.: Reps. Health. Dep. Panama Canal, 1919-1920.

205. Bascom, G. R., in Rep. Div. Sanitation, S. G. O., quoted by Craig: in Chapter on Malaria in Med. Dept., U. S. A. in World War, Govt. Print. Off., Washington, D. C., 1928, vol. 9.

experiments were very extensive and conclusive. He caught and stained mosquitoes, liberating them at various distances from the camp, and then recaught as many as possible. During the time covered by the experiments there were only slight air currents, so that the distances covered by the mosquitoes could not be explained by their being carried by winds. More than 5,000 mosquitoes were experimented with and recatching was done at thirty-seven different stations. It was found that the distance covered by the flight of the anophelines varied all the way from one quarter of a mile to two and one half miles, the greatest number of mosquitoes being caught at a distance of from one-half to one and one quarter miles. His conclusion was that in the region in which the experiments were performed a flight distance of at least one mile could be expected, but the range of flight of *Anopheles quadrimaculatus,* the most common mosquito present, and used in the experiments, could be as much as two and one-half miles.

In Macedonia, Wenyon found that the length of flight of anopheline mosquitoes varied greatly but he states:

> The range of flight, especially when it can be done in stages, is not half a mile, but often two or three miles or even more. The absence of a population near a breeding-place encourages the mosquitoes to travel long distances to find a host upon which to feed. The result is that the camps, though surrounded by a cleared area of half a mile, even if this area were properly maintained, became the center of attraction for mosquitoes breeding all over the country.

*Mosquito Species Control of Malaria.*—As already stated, modern prophylactic measures against malaria, so far as mosquitoes are concerned, depend on the destruction of the special species of anopheline mosquitoes that are the most efficient transmitters of malarial infection. This aspect of prophylaxis has been stressed in this country, especially by Darling.[206] He was one of the first to call attention to the importance of limiting prophylactic measures, so far as mosquitoes are concerned, to those affecting the principal transmitters, but his observations have been confirmed by all field workers in malaria prophylaxis.

*Destruction of House Mosquitoes.*—The catching and killing of anopheline mosquitoes in houses in malarial regions has recently received an impetus from the observations and recommendations of James,[15] but the value of this measure was first urged by Orenstein,[207] in the Canal Zone, who reported excellent results in prophylaxis against malaria by the use of this method. The importance of this method was generally overlooked until the recent statements of James that malaria is largely a house disease transmitted by anophelines living in the house and infecting the inhabitants. He calls the human habitation the "labora-

206. Darling, S. T.: Am. J. Trop. Dis. **6**:167, 1926.

207. Orenstein, A. J.: Am. J. Pub. Health **3**:106, 1913.

tory" of malaria and believes that the destruction of these house mosquitoes with the detection and treatment of "carriers" of the plasmodia constitute the most important prophylactic measures available for the prevention of malaria. The opinion of James regarding the relative importance of the destruction of mosquitoes in houses in the prophylaxis against malaria is not shared by the majority of malariologists, and time alone will determine the question, but there is no doubt that the catching of anopheline mosquitoes in houses is a most important prophylactic measure.

*Animal Prophylaxis.*—Many observers have noted that mosquitoes feed on domestic animals and it has been recently ascertained that certain species transmitting malaria prefer the blood of certain animals to that of man. Thus, Iyengar [208] classifies the anopheles of India into classes with reference to this subject. Omitting essentially wild species, he places all Indian anopheles in three classes: (1) Species preferring the blood of animals to that of man—*Anopheles hyrcanus* (*sinensis*), *fuliginosus, jamesi, maculipalpis* and *maculatus;* (2) species preferring the blood of man to that of animals—*Anopheles vagus, subpictus, jeyporensis* and *culicifacies;* (3) species living solely on human blood—*Anopheles minimus, listoni, varuna* and *stephensi.* Similar observations have been made elsewhere and suggestions have been made that this fact may be useful in prophylaxis and Roubaud and others have urged the value of cattle stables near houses in malarial regions in deflecting the mosquitoes from man and thus lessening the chances of infection. Swellengrebel,[209] Roukhadze [210] and Hehir [200] admit the effect of animals in reducing malaria in certain regions and the latter writer states that the vast number of domestic animals in India may have had something to do with keeping malaria within bounds in that country, but he is not satisfied that the effect of animal screens in diverting mosquitoes from man has been sufficiently investigated to warrant expensive measures based on this theory. Roubaud and Leger [211] first attracted attention to the use of domestic animals in prophylaxis against malaria, and Roubaud states that the protective value of this method consists in the dilution of the anopheline infection, the dilution of the human infection and progressive sterilization of infected anophelines through the destruction of the sporozoites injected into animals.

The subject of the value of animal screens in the prophylaxis against malaria has not been extensively investigated in this country, and it is doubtful if it should be seriously considered as a prophylactic method in most regions, for other and better methods are available.

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208. Iyengar, M. O. T.: Rep. Proc. 5th Ent. Meeting at Pusa, 1923, p. 203.
209. Swellengrebel, N. H.: Bull. Soc. path. Exot. **18**:725, 1925.
210. Roukhadze, N.: Bull. Soc. path. Exot. **19**:100, 1926.
211. Roubaud and Leger: Trop. Dis. Bull. **18**:320, 1921.

A method of considerable importance in the destruction of mosquitoes has been recently added to prophylactic resources in the use of Paris green as a larvicide. This agent has been extensively used in the United States and other countries and has been found an efficient larvicide. It is used in the proportion of 1 part of Paris green to 100 parts of sand, fine ashes, etc., and is sprayed on the surface of the breeding places of mosquitoes. In this proportion it is not harmful to animals or man, and the results obtained with it have been most excellent. In the United States, large areas have been sprayed with this larvicide by means of the aeroplane and this method of distributing the agent enables one to treat swamps and other collections of water that are inaccessible by ordinary methods of transportation.

*The Protection of Man from the Bites of Mosquitoes.*—The protection of man from the bites of mosquitoes is a method of prophylaxis that is usually necessary in conjunction with the methods for destroying mosquitoes, as it is rarely that all mosquitoes can be destroyed in practice. This method depends essentially on the use of proper screening for human habitations, the use of mosquito nets for the bed, and of headnets and gloves for those who are compelled to be exposed to bites of these insects. The methods of screening habitations are thoroughly described in all works on malaria and prophylaxis against malaria, as well as the proper methods of using mosquito nets, headnets, etc., and will not be considered here. The most important factor in the success of this method of prophylaxis is the mesh of the netting used in the screening, and observations made within comparatively recent years have added to the knowledge of this subject. In 1909, as the result of observations made in the Philippine Islands, I [212] concluded that screening containing sixteen meshes to the linear inch was efficient in excluding all species of anophelines present in those islands. These observations were confirmed by Guiteras,[213] Darling [86] and the United States Army Board for the study of Tropical Diseases in the Philippines.[214] Guiteras reported, as the result of careful experiments, that no. 16 (sixteen meshes to linear inch) netting was small enough to exclude all anophelines and also the yellow fever and dengue mosquito, *Aedes aegypti,* but Darling found that while no. 16 netting was practically safe, so far as *Aedes aegypti* was concerned, it was not absolutely so, as some of the smaller individuals of this species were able to get through this netting, as well as some species of Culex, but that it excluded all anophelines. The United States Army Board for the Study of Tropical Diseases found

212. Craig, C. F. (footnote 21, third reference).

213. Guiteras, J., quoted in Howard, Dyar and Knab: The Mosquitoes of North, Central and South America, Washington, D. C., Carnegie Institute.

214. U. S. Army Board, etc., Rep. Surg.—Gen. Army, Govt. Print. Off., Washington, D. C.

that no. 16 netting excluded all anophelines in the Philippines, confirming my conclusions, but that it did not exclude the yellow fever and dengue mosquito, *Aedes aegypti,* and, as the result of their experiments, recommended the use of no. 18 mesh netting which excluded all mosquitoes that were tested.

Where it is desired to screen only against anopheline mosquitoes a netting containing sixteen meshes to the linear inch (no. 16) will be safe in protecting against malarial infection, but in regions in which either dengue fever or yellow fever occur the netting used should contain 18 meshes to the linear inch (no. 18 netting).

*The Destruction of the Malaria Plasmodia in Man.*—This important method of prophylaxis against malaria depends on destroying the malaria plasmodia in the human host, either before or after the development of symptoms of the infection. As the most efficient agent for accomplishing this purpose is quinine, or other derivatives of cinchona, the method is broadly known as "quinine prophylaxis" but, as will be seen later, in view of modern knowledge of the action of quinine, it would better be known as "quinine prophylactic treatment." None of the methods of prophylaxis against malaria have caused more controversy than the use of quinine. Enthusiasts in its favor have claimed that the proper use of this drug alone is all that is necessary to eradicate the plasmodia from any locality, and, therefore, cause the disappearance of malaria, while its most violent enemies have claimed that it is not only useless but actually harmful. As a matter of fact, the truth lies between these extreme opinions and there is no doubt, that properly administered and in sufficient dosage, quinine is useful as a prophylactic against malarial infections.

*Action of Quinine on the Malaria Plasmodia.*—In considering this subject the action of quinine on the forms of the plasmodia occurring in the blood of the human host and of those occurring in the transmitting mosquito must be discussed, for recent observations show that the action of the drug on the forms occurring in the asexual and sexual cycle of development of the parasites differs markedly.

*a.* Action of Quinine on Human Life Cycle Forms: The observations of many investigators have demonstrated that on certain of the asexual forms of the malaria plasmodia occurring in the blood of man, quinine appears to have a marked action and that it is capable, directly or indirectly, of destroying certain of these forms. In 1919, I [121] described in detail the action of quinine on *Plasmodium vivax, Plasmodium malariae* and *Plasmodium falciparum,* my studies being based on changes in morphology occurring in these parasites after the administration of the drug as shown in both living and stained preparations of the parasites. The staining reactions with Wright's stain remain

the same with the exception that the chromatin stains a dark red or almost black instead of the normal ruby red, after therapeutic doses of the drug. The effect of quinine was shown to consist in an injury to the plasmodia that prevented the normal development of the schizont by preventing multiplication of the chromatin, so that the segmenting forms contained fewer segments, or merozoites, than usual and many of these were found to be devoid of chromatin. The drug also caused shrinkage of the cytoplasm and fragmentation of many of the plasmodia, so that the number of the parasites became markedly reduced within a day or two after the beginning of the administration of the drug. The effect of the drug appeared to be most marked on the merozoites and on the schizonts up to the time of three-quarters growth, although the fully developed schizonts often showed signs of fragmentation. The fragmentation of the younger schizonts and the extrusion of the chromatin were the characteristic evidences of the destruction of the parasites and were frequently noted, while those parasites that continued to live developed in an atypical manner and the number of merozoites produced was reduced and many of them did not contain chromatin.

These observations, which confirmed and extended those of Golgi, Romanowsky, Ziemann, Mannaberg, Marchiafava and Bignami and others, demonstrate that quinine adversely affects the development of the human life cycle forms of the malaria plasmodia, but whether this effect is due to a direct action on the parasites, to changes brought about in the red blood corpuscles rendering them unsuitable for the normal development of the plasmodia or to a metabolite produced by the drug in the human body is still an open question. Yorke and Macfie [65] believe that quinine destroys directly a certain number of the plasmodia and that these set free antigen which stimulates the formation of antibodies and that thus the infection is destroyed; while the experiments of Mühlens and Kirschbaum [12] appear to negative the opinion that quinine acts directly on the plasmodia. They found that mixing blood containing the malaria plasmodia with a solution of quinine hydrochloride (1:10,000), and keeping this mixture for twelve hours, did not destroy the infectivity of the blood to man, and if the strength of the quinine was increased to 1:5,000, the blood was still infective after five hours. Lipkin [215] explains the curative action of quinine by the suggestion that a plasmodicidal metabolite is formed in the body from quinine, but no evidence that this is true has been adduced. However, whatever the explanation of the action of quinine on the human life cycle forms, it is not questionable that this drug does, either directly or indirectly, destroy the malaria plasmodia in the blood of man.

215. Lipkin: Ann. Trop. Med. **19**:149, 1919.

The action of quinine on the gametocytes of the plasmodia in the blood of man has already been considered in the discussion of the factors pertaining to the transmission of malaria by the mosquito, and it was noted that Mayne [216] and Wenyon [98] had shown that anopheles mosquitoes could be infected with *Plasmodium falciparum* from patients taking quinine, but that infection of the mosquitoes rarely followed in the case of *Plasmodium vivax*. Barducci [93] found that patients taking quinine and whose blood contained *Plasmodium vivax*, *Plasmodium malariae* or *Plasmodium falciparum*, were not infective to *Anopheles maculipennis* as the gametocytes failed to complete their life cycle in the insects and no sporozoites were produced. It is, therefore, apparently true that quinine not only destroys the human life cycle forms in the blood of man, but so affects the sexual forms (gametocytes) that they cannot develop to maturity in the transmitting mosquitoes, thus acting as a prophylactic in both man and the mosquito.

The reduction in the ratio of infected mosquitoes in malarial regions after the mass treatment of the inhabitants with quinine has been noted by numerous observers, and recently Vestschezerow [104] contributed a significant paper on this subject. He found that in the Caucasus region around the Black Sea no less than 20 per cent of 194 *Anopheles maculipennis* caught in sleeping quarters were infected with the malaria plasmodia, but that after one month's treatment of the inhabitants with quinine the percentage of infected mosquitoes had been reduced to 7.14 per cent.

*b.* Action of Quinine on Mosquito Life Cycle Forms: The action of quinine in preventing the development of gametocytes in the mosquito has just been considered, and the question now arises, does quinine have any action on the sporozoites that are introduced into man when the insect bites? If it can destroy the sporozoites it is evident that the drug can prevent malarial infection, but if it cannot, infection will occur although the drug may prevent the development of symptoms of the infection. Recent studies have shown definitely that quinine in the amounts present in the blood after prophylactic doses, has little, if any, destructive effect on the sporozoites. James, Nicol and Shute,[217] having demonstrated that the injection into man of sporozoites obtained from infected mosquitoes produced infection, performed the following experiment: Two mosquitoes infected with *Plasmodium vivax* were placed in a 1 : 5,000 solution of quinine bisulphate made up with human blood serum, and their salivary glands dissected and teased up in the solution. One cubic centimeter of this solution was then drawn

216. Mayne, B.: U. S. Pub. Health Rep. **35**:1664, 1920.

217. James; Nicol and Shute: Tr. Roy. Soc. Trop. Med. & Hyg. **21**:233, 1927.

into a hypodermic syringe, allowed to remain for fifteen minutes, and then injected subcutaneously into a patient. At the end of ten days, the patient had a malarial paroxysm and *Plasmodium vivax* was found in the blood. This experiment apparently proves that the sporozoites of *Plasmodium vivax* exposed for fifteen minutes to a concentration of quinine equal to 15 grains (1 Gm.) in the human being are not injured so far as infectivity is concerned, and that this dosage of quinine in prophylaxis is not sufficient to prevent infection.

That this conclusion is justified has been borne out by actual field experience and by experimental evidence, for it has been shown by several investigators that infection with malaria does occur even though the individual is taking as much as 1 Gm. of quinine a day.

*The Use of Quinine in the Prophylaxis Against Malaria.*—From the foregoing observations it would appear that quinine, either directly or indirectly, destroys the human life cycle forms of the malaria plasmodia in the blood of the human host, but has no effect on the sporozoites. It is thus evident that, strictly speaking, quinine cannot prevent infection from the mosquito as it does not kill the sporozoites when in the blood of man in the concentration usually employed in prophylaxis. Although this is true, and infection cannot be prevented, even though as much as 1 Gm. of quinine be taken daily, it is also true that this amount of the drug will prevent the appearance of symptoms of malarial infection as long as it is being taken, and therapeutic doses will cure the infection even before the development of symptoms. In his extensive observations in the treatment of paretic patients by the induction of malaria, Yorke [218] states that quinine does not destroy the sporozoites, but that daily doses of 10 grains (0.65 Gm.) of the drug in solution continued for ten or more days after the infective mosquito bites prevents the development of symptoms of the infection by destroying the trophozoites, the first stage in the development of the plasmodia in man. Yorke and Macfie [65] believe that cure of the infection is brought about in the following manner: The quinine, by destroying large numbers of the plasmodia, directly or indirectly, sets free antigenic substances which stimulate the production of specific antibodies, which, if sufficient in amount, destroy the remaining plasmodia, thus resulting in the cure of the patient. On the other hand, Giemsa [219] believes that it must be accepted that quinine acts directly on the plasmodia, destroying them, and that it is not capable of releasing specific immune bodies which, however, may be present in the blood and may aid the quinine in overcoming the infection, the immune body being produced as the result of the destruction of the plasmodia by quinine.

218. Yorke, W.: Tr. Roy. Soc. Trop. Med. & Hyg. **19**:108, 1923.
219. Giemsa, G.: München. med. Wchnschr. **74**:574, 1927.

A most excellent contribution to knowledge of the effect of quinine in prophylaxis against malaria has been published recently by McNabb and Stewart [220] (1927), officers of the Medical Corps of the United States Army, on duty in the Canal Zone. Their observations were made on three companies of United States Army Engineers, employed in mapping Panama. Quinine was compulsory and 1 Gm. was given each soldier daily in tablets or capsules before the evening meal, under strict supervision. The men worked and were camped in intensely malarial localities and near natives who were undoubtedly carriers of the infection. They found that of 225 United States Engineers engaged for four and a half months in mapping unsanitated sections of the Isthmus of Panama and taking 1 Gm. of quinine daily, only 14, or 6 per cent, developed clinical symptoms of malaria and that only after quinine was discontinued did the actual amount of infection become manifest by the appearance of symptoms in 106 persons, or 47 per cent of the command. They thus confirm Yorke and Macfie's observation that quinine does not prevent malarial infection, but were unable to confirm these observers in their finding that continued use of the drug for two weeks after exposure resulted in the prevention of clinical malaria. As a reason for this they cite massive infection, climatic conditions and physical hardship, and state that it is possible that some who did not show symptoms after stopping quinine were also infected, but were cured by the daily use of the drug. They conclude that under field conditions 1 Gm. of quinine will prevent the development of symptoms of malaria in men exposed to infection and will keep them on duty, although it will not prevent infection, and that "quinine prophylaxis," therefore, while defective in the strict sense of the word, has a great military value, and will enable troops to accomplish a mission in a malarious region. They also found that estivo-autumnal malaria is easier to prevent than tertian.

The observations of McNabb and Stewart demonstrate that quinine in the dosage given did not prevent malarial infection in 47 per cent of the persons concerned, but the fact that over 50 per cent of these persons lived and worked for four and a half months in most malarious regions and did not develop the symptoms of malaria, even after discontinuing the use of quinine, indicates that even though infection may not have been prevented, a considerable number of persons must have been cured of the infection, if it did occur. It is impossible to believe that over 50 per cent of a command could escape the development of clinical malaria had quinine not been administered.

The effect of a larger prophylactic dose of quinine than 1 Gm. should be investigated, for it is probable that, in the light of the observa-

220. McNabb and Stewart: Am. J. Trop. Med. **7**:357, 1927.

tions of McNabb and Stewart, a dose of from 1.3 to 1.5 Gm. (20 to 25 grains) would be successful in curing the vast majority of the infections that might occur, before the development of symptoms. Such doses of quinine can be taken for long periods of time without injury or much discomfort, as has been shown repeatedly, and one should not hesitate to employ them in very malarial regions. Whatever may be thought of the value of quinine as a prophylactic in the actual prevention of malaria, there is no question of its value in the prevention of the disease by its therapeutic use in the treatment of carriers of the plasmodia and of latent and initial infections.

*Discovery and Treatment of Carriers.*—A "carrier" of malaria is one who has gametocytes of the plasmodia in his blood in sufficient number to infect mosquitoes, and the great importance of the discovery and treatment of these carriers has only recently been emphasized, although in 1906,[105] in my studies of this subject in the Philippines, I stated that in certain regions, as Camp Stotsenberg, the discovery and treatment of the native "carrier" was the only method of solving the problem of malarial transmission in that locality. Within recent years this subject has attracted more attention, and it is obvious that if carriers of malaria can be discovered and rid of the gametocytes by proper treatment, mosquitoes will not become infected and malaria will disappear. It has been shown that carriers can be rendered harmless by proper treatment, and that it is feasible to employ this method in prophylaxis. While it is possible to prevent the development of gametocytes by the proper treatment in initial malarial infections, it is much more difficult to rid the patients of these forms of the plasmodia after they have once appeared in the blood. Indeed, the consensus of modern opinion and research on this subject is that quinine does not have any effect on the gametocytes, but causes their disappearance by destroying the merozoites or the schizonts from which the gametocytes are derived. Thus no more gametocytes are produced; those present in the blood gradually die of old age, and the patient becomes noninfective to the mosquito.

The proper treatment of carriers of malaria plasmodia depends on their recognition. This is only possible by microscopic examination of the blood of persons having malarial infections. As gametocytes are found not only in those having definite clinical symptoms of malaria but also in latent infections in persons apparently in good health and symptomless, it follows that the discovery of carriers, if it is to be successful, means the microscopic examination of the blood of all persons in malarial regions. In practice, this is frequently impossible owing to the number of examinations necessary and to the cost, but in small communities it is possible, and in the instances of labor camps and settlements, military camps and all collections of

people under discipline and executive administration, it is generally possible to make such a survey and to treat properly those who have gametocytes in their blood. In the light of the recent observation that if quinine is present in the blood at the same time that gametocytes are, mosquitoes will not become infected with malaria because the gametocytes will not mature in the mosquitoes under such conditions, it would appear that it is not necessary to segregate and screen carriers of malaria. Such persons should report daily for treatment with quinine, and after receiving it may go about their business as usual. I believe that further research is necessary before the harmlessness of carriers whose blood contains quinine in therapeutic doses can be accepted as proved, and that the evidence is insufficient to warrant the treatment of such carriers with quinine without segregating or screening them.

It has been shown by many observers that gametocytes disappear from the blood of carriers after proper treatment by quinine, and that such treatment has to be controlled by the microscopic examination of the blood is self-evident. The administration of from 1.3 to 2 Gm. (20 to 30 grains) of quinine sulphate by mouth for a period of from three to four weeks is usually followed by the disappearance of gametocytes from the blood and the cure of the malarial infection. Smaller doses of quinine have been advocated by Bass and others, and Bass has secured excellent results in antimalarial work in Mississippi by the daily administration of 65 cg. of quinine sulphate.

The recent introduction of a quinolin derivative, known commercially as "plasmochin," has apparently added a valuable aid to the physician and health officer in the fight against malaria. The many observations that have been made regarding the action of this drug have definitely shown that it acts directly on the gametocytes, causing their disappearance from the blood within a few days, and that it is much more efficient in the treatment of carriers than any of the salts of quinine, so far as causing the disappearance of gametocytes is concerned. Its use in the prophylaxis against malaria, under medical supervision, should be encouraged and the results carefully noted. This drug will be more thoroughly discussed in the section of this review dealing with the treatment of malarial infections.

*Treatment of Patients with Initial and Recurrent Malarial Infections.*—The proper treatment of patients with initial malarial infection is a most important prophylactic measure, for if such patients are so treated, gametocytes will never develop, and thus the infected persons will never become infective to mosquitoes. As already noted, gametocytes do not develop until a malarial infection has persisted for several days, and if persons with this infection are properly treated with quinine from the beginning of the clinical symptoms, gametocytes will never be found in the peripheral blood. Therefore, it follows

that every person in any community whose blood contains gametocytes is an evidence of either improper treatment, or of no treatment, and if of improper treatment, an evidence of the ignorance or carelessness of the attending physician. As I have stated before:[188]

> It is a sad fact that a very large proportion of the malaria present in most localities is directly due to the improper treatment of malarial infections coming under the direct care of medical practioners, because of the common practice of regarding such infections as cured when the symptoms disappear, the patient being allowed to resume his work without any direction regarding the continued use of quinine.

The prompt recognition, through an examination of the blood, of malarial infection and the prompt and thorough treatment of the patient will prevent the appearance of gametocytes in the blood in cases of initial infections with the plasmodia, and will thus prevent the infection of the mosquito; this is a most important duty of the physician in all malarial localities.

The proper treatment in recurrent attacks of malaria is also important as regards prophylaxis. Many of the patients with a relapsing infection already have gametocytes in their blood at the time they are first seen by the physician, and are thus already carriers of malaria. Such patients should be given the treatment already outlined for carriers, but those patients with recurrent infections in whom gametocytes are not present should be treated in the following manner:

The method of treatment in cases of initial malarial infections and in those of recurrent infections in which gametocytes are not present, varies with the severity of the symptoms. During the acute attack, while symptoms are present, 2 Gm. of quinine sulphate should be given daily in divided doses, and after the disappearance of the symptoms at least 1 Gm. of quinine sulphate should be administered daily for at least three months, either in one dose at night or in a divided dose morning and evening. Some authorities recommend a daily dose of 65 cg. for the same period of time, but I believe that better results are obtained with the larger dose. In the cases of relapsing infection, if gametocytes are present, the treatment should be that already described for carriers of malaria.

*Discovery and Treatment of Latent Malarial Infections.*—It has already been noted that in all malarial regions the examination of the blood of the natives will show a varying percentage of persons whose blood is positive for the malaria plasmodia, but who do not have clinical symptoms of the infection. The discovery and treatment of these cases of latent malarial infection is most important as regards prophylaxis, as many of the patients have gametocytes in their blood and are carriers of malaria, while those that have not may develop gametocytes at any time and become carriers.

The percentage of latent malaria in malarial regions varies greatly, but innumerable observations show that the percentage is generally high in children between the ages of from 1 to 10 years, and lower in adults, although many regions are known in which the adult percentage is as high as that of children. To Koch [221] belongs the credit of first calling attention to the occurrence of latent malaria in the natives of malarial regions, and his observations on this subject demonstrated that in the regions he investigated in Africa, the children were almost invariably infected, and that many of them did not show symptoms of the infection. Plehn,[222] in the Kameruns, found that 97 per cent of the children and 60 per cent of adults examined harbored malaria plasmodia. Various other authors have found the following percentage of infection: Olwig,[223] in Dutch East Africa, 35 per cent of children and 16 per cent of adults; Craig,[105] at Camp Stotsenberg, in the Island of Luzon, P.I., 75 per cent of children from 1 to 5 years of age, and 62 per cent of adults; Thomas,[224] in Manaos, Brazil, 50 per cent of children; the Sergents,[102] in Algeria, 25 per cent of children and 33 per cent of adults; Sorel,[225] on the Ivory Coast, Africa, 55 per cent of children and 43 per cent of adults. Bass [226] records the results of the examination of the blood of 31,459 persons in Mississippi for malaria plasmodia of whom 6,664, or 21.18 per cent, were found positive. The rate of infection under 20 years of age was 23.56 per cent, while the rate of infection over 20 years of age was 19.22 per cent. Of the 6,664 infected persons, 3,671, or 55.09 per cent, gave a history of a malarial attack within one year previous to the examination, while 2,993 gave a negative history as regards malarial attacks. In contradistinction to these results of Bass, in which the rates for children and young adults and for older adults were almost equal, are the results of Mansfield-Anders,[85] in Zanzibar, who found *Plasmodia* in the blood of 67.8 per cent of 1,542 children and in that of only 20.6 per cent of 479 adults. It is thus evident that the percentage of latent malarial infection and the relative incidence in children and adults, varies greatly in different localities and that where malaria is most severe the rate in children is higher than that in adults, probably owing to the immunity acquired by the adult from numerous infections during childhood.

The percentage of carriers among the latent malarial cases also varies in different regions, but it is the consensus of opinion of most

221. Koch, R.: Deutsche. med. Wchnschr. **5**:88, 1900.
222. Plehn, A.: Virchows Arch. f. path. Anat., supplement, 1903.
223. Olwig, R.: Ztschr. f. Hyg. u. Infec. **45**:345, 1903.
224. Thomas, H. W.: Ann. Trop. Med. **4**:7, 1910.
225. Sorel, F.: Bull. Soc. path. Exot. **10**:365, 1912.
226. Bass, C. C.: Southern M. J. **12**:190, 306 and 456, 1919.

malariologists that from 40 to 50 per cent of the persons with latent infection have gametocytes in their blood and are carriers of malaria. In regions in which the malarial index is high, the percentage of carriers is usually even higher than 50 per cent.

The discovery of persons with latent malarial infections (which can only be made by a microscopic examination of the blood) and their proper treatment is of great importance in the prophylaxis against malaria, and is a method advocated by all authorities, so far as it is practicable. If gametocytes are not present, the best treatment in cases of latent infection is the administration of 2 Gm. of quinine daily until the plasmodia disappear from the blood, which is generally within from three to five days. The drug is best given in three equally divided doses, morning, noon and at night. After the plasmodia have disappeared, the administration of 1 Gm. daily for two weeks and of 65 cg. daily for a month or two afterward, has been found efficient in curing these infections.

If gametocytes are present, the treatment is the same as that for carriers which has already been discussed.

The relative value of the various methods of prophylaxis against malaria that have been reviewed differs in different localities and no one method can be said to be the most valuable one under every natural condition. Castellani [227] collected some valuable data regarding this subject during the World War. The data covered the results of prophylaxis against malaria in four different camps, all located in endemic malarial regions which were similar as regards the intensity of the malarial infection. In Camp no. 1, no antimalarial measures were employed and almost 100 per cent of the officers and men developed clinical malaria. In Camp no. 2, prophylaxis with quinine alone was employed, and in this camp 45 per cent of the personnel developed malaria, a protection rate of 55 per cent being obtained. In Camp no. 3, antimosquito measures alone were employed, and 25 per cent of the personnel developed malaria, a protection rate of 75 per cent. In Camp no. 4, both antimosquito measures and prophylaxis with quinine were employed, and in this camp only 6 per cent of the personnel developed malaria, a protection rate of 94 per cent.

The results recorded by Castellani are borne out in field work in the prophylaxis against malarial infection and demonstrate that the greatest measure of success is obtained when antimosquito measures are combined with the prophylactic use of quinine. Under certain conditions, as in military operations, the use of a certain method of prophylaxis may give much better results than other methods. Thus, Wenyon [95] found that in Macedonia the protection of the English

227. Castellani, A.: Personal communication, 1926.

troops from the bites of mosquitoes was the most valuable method of prophylaxis adopted and says: "Mosquito nets did more to prevent infection than all the other methods of prevention of malaria together."

Within recent years, an immense amount of data has been accumulated regarding prophylaxis against malaria; it has been shown that no one method is suitable under all conditions, but that the subject must be approached from a broad standpoint and the methods adopted that are most suitable to the local conditions present. While, in rare instances, it may be possible to eradicate these infections by the use of one method, as the destruction of the breeding places of transmitting mosquitoes, it has been found that in the vast majority of cases the best results have been obtained by the use of combined methods of prophylaxis.

### TREATMENT

Within recent years, researches on the treatment of malarial infections have been devoted largely to the study of the various derivatives of cinchona bark, and of drugs that have been advocated, from time to time, as substitutes for quinine in the treatment of these infections. As the methods of using quinine in the treatment of patients with malaria are fully discussed in all works on the malarial fevers, they will not be considered here, beyond what has already been stated regarding the use of the drug in prophylaxis. No amount of criticism or adverse opinion affects in the least the vast amount of clinical evidence that proves that quinine, directly or indirectly, cures malarial infections, and the efforts that have been made within recent years to discredit this drug as a specific for these infections have only served to emphasize its value in treatment and to discredit those who have been responsible for such efforts.

*Relative Merits of Different Preparations of Cinchona.*—Much data has been accumulated within recent years relating to the relative value of different crystallizable alkaloids of cinchona and of quinine sulphate, the most largely used of all the derivatives of cinchona bark. MacGilchrist [228] tested the therapeutic value of the various alkaloids obtained from cinchona on prisoners in India by observing the time elapsing between the administration of the first dose and the disappearance of the asexual forms of the plasmodia from the peripheral blood. His results indicated that the various alkaloids stood in the following order as regards their power to remove the plasmodia from the peripheral blood: (1) hydroquinine hydrochloride; (2) cinchonine sulphate; (3) quinine sulphate; (4) quinidine sulphate; (5) ethylhydrocupreine hydrochloride; (6) cinchonidine sulphate, and (7) quinoidine. Of these, cinchonine and quinoidine frequently caused vomiting and

228. MacGilchrist, A. C.: Indian J. M. Research **3**:1, 1915.

ethylhydrocupreine hydrochloride may cause optic atrophy. In 1920, Acton,[229] using *Paramoecium caudatum* for testing the action of the cinchona alkaloids on protozoa, found that they were toxic in the following order: ethylhydrocupreidine, ethylhydrocupreine hydrochloride, quinidine, cinchonidine, quinine and cinchonine. James,[230] as a result of his experience, states that the choice of alkaloids lies between hydroquinine, quinine and quinidine, and Knowles and Senior-White[201] state that it is far from certain that quinine is the best alkaloid of cinchona to use in the treatment of persons with malaria as both quinidine and cinchonidine are more powerful in their action as antimalarial agents. The Malaria Commission of the League of Nations[231] reports that quinine and quinidine are nearly equally effective in doses of 1 Gm. daily, while cinchonine requires a daily dosage of 1.5 Gm. to be as effective as either quinine or quinidine. The Commission also reports that an extract of the total alkaloids of cinchona, as in the renowned "cinchona febrifuge," is as effective as quinine in curing malaria.

Cinchona febrifuge has been urged by Acton and Knowles[232] as a substitute for quinine, as it is as efficacious as quinine in the treatment of persons with malaria and is much cheaper. Cinchona febrifuge is used extensively in India and contains several of the alkaloids of cinchona. Its composition, according to MacGilchrist,[233] is as follows: quinine, 7.4 per cent; quinidine, 22.83 per cent; cinchonine, 18.58 per cent; cinchonidine, 5.84 per cent; uncrystallizable alkaloids, ash, etc., 43.35 per cent. Knowles and Senior-White recommend the employment of this mixture of the alkaloids, but state that it is apt to cause vomiting unless taken not less than two and one-half hours after eating. Fletcher[234] states that in his experience, quinine febrifuge, given in doses of 10 grains daily, gave as good results in the treatment of persons with malaria as the same amount of quinine and was not more toxic, but that given in larger doses, as 0.1 grain per kilogram of body weight, it was not as satisfactory as quinine, and still larger doses were much more toxic than quinine.

Quinine febrifuge was originally a combination of all the alkaloids and active principles extracted from cinchona bark, but as now made

229. Acton, H. W.: Indian J. M. Research **9**:339, 1920.

230. James, S. P., in Byam and Archibald: Practice of Medicine in the Tropics, New York, Oxford University Press, 1922, vol. 2, p. 1568.

231. Malaria Committee, League of Nations; abst., Trop. Dis. Bull. **25**:115, 1927.

232. Acton and Knowles: Indian M. Gaz. **59**:177, 1924.

233. MacGilchrist, A. C.: Indian J. Med. Research **3**:315, 1915.

234. Fletcher, W.: Notes on the Treatment of Malaria with the Alkaloids of Cinchona, London, John Bale Sons & Danielsson, 1923.

in India consists of the residual alkaloids after the quinine has been extracted, quinine being added in quantity sufficient to make the mixture like the original extract. It is apt to be grossly adulterated and often consists simply of the by-products obtained in the manufacture of quinine, so that the only safe "quinine febrifuge" is that manufactured under government supervision. It is issued from the quinine factories of the government of India in powder form and in tablets. It is best given in tablet form. As this product is much more cheaply prepared than is quinine sulphate, its use should be favored provided it can be standardized from a pharmaceutical standpoint and manufactured under a government permit.

While it is undoubtedly true that quinidine and cinchonidine are more powerful antiplasmodial drugs than quinine sulphate, the latter salt remains the favorite in the treatment of persons with malaria because of its relative cheapness, effectiveness and availability. The efforts that have been made to popularize the other alkaloids of cinchona, or quinine febrifuge, have not met with any great success except in limited localities.

*Quinine and Alkalies.*—According to Sinton [235] and others, there is a tendency to acidosis in malaria, and the administration of alkalies with quinine has been urged by this observer and by Knowles and Senior-White.[201] The experiments of Acton and Knowles [232] showed that quinine combined with alkalies was more fatal to *Paramoecium caudatum* than quinine alone, and on these experiments is based the argument for the use of alkalies with quinine in malaria. More recent observations do not appear to support the belief and the question is still unsettled. I have never found it necessary to add alkalies to quinine in order to secure good results in the treatment of persons with malaria, and it has not been found necessary by the vast majority of practitioners in malarial regions.

*Methods of Administration.*—Recent observations have not added any data of great importance as to the relative value of different methods of administration of quinine in the treatment of patients with malarial infections. Oral administration is still the method of choice in cases of the usual malarial infection, and experience has only confirmed the great value of intravenous administration in severe and pernicious cases of malaria. The old prejudice against the administration of tablets has been overcome because at the present time soluble tablets are always available, but in time of great demand, as in war, it is still believed that the administration of quinine in tablet form should be carefully controlled and should be replaced by quinine in solution when possible.

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235. Sinton, J. A.: Indian J. M. Research **11**:1051, 1924.

The intramuscular administration of quinine, which became exceedingly popular with English medical officers during the World War, has been carefully investigated by several observers and the consensus of opinion now is that this method should not be used as a routine method of administration of the drug but be reserved for carefully selected cases.

Fletcher [234] has shown by a series of careful experiments that the intramuscular injection of quinine invariably causes necrosis of the tissue into which the drug is injected, and while this necrosis may be so slight as not to give rise to definite symptoms, it is a fact that severe necrosis and widespread destruction of tissue and function not infrequently follow this method of administration. Fletcher's observations have been confirmed by Bassett-Smith,[236] Acton and Knowles [232] and Knowles and Senior-White,[201] and McLay [75] has shown that the malarial plasmodia disappear more quickly from the peripheral blood after oral administration of quinine than after intramuscular injection. Knowles and Senior-White state that "all of the leading authorities in tropical medicine in England are opposed to this practice," and it may be added that this statement is true of all such authorities in the United States. Intramuscular injection of quinine should be reserved for those cases of malaria in which administration by mouth is impossible, for young children, or for patients to whom it is impossible to administer the drug intravenously. In severe malarial infections, in which the symptoms are pernicious, intravenous injection of quinine should always replace intramuscular injection, in the opinion of practically all malariologists.

*The Absorption of Quinine.*—The efficacy of the treatment of persons with malaria with quinine depends on the absorption of the drug. It has been shown in recent years that nonabsorption of quinine is of frequent occurrence, and the value of testing for the absorption of the drug, as a guide in treatment, has been emphasized. The many criticisms in the recent literature regarding the inefficacy of treatment with quinine in cases of malaria would have been greatly reduced, and perhaps entirely eliminated, had the critic been sure that the quinine administered was actually absorbed by the patient. The absorption of this drug can be easily and readily detected by the use of Mayer's reagent, which consists of 13.55 Gm. of mercuric chloride and 49.8 Gm. of potassium iodide in 1 liter of distilled water, and which is employed as follows:

The patient's urine, filtered to rid it of phosphates, is examined for albumin, which, if present, is removed by adding acetic acid, bringing slowly to a boil, and filtering. Five cubic centimeters of the filtered

236. Bassett-Smith, P. W.: J. Roy. Nav. Med. Serv. **9**:126, 1923.

urine are placed in a test tube and from 3 to 4 drops of the Mayer reagent is added. If the quinine has been absorbed, it will cause a cloudiness when the Mayer reagent is added. If the urine contains other alkaloids, as strychnine, atropine, etc., it will also show a cloudiness with this reagent, so that this fact should be ascertained before applying the test.

The most common cause of the nonabsorption of prescribed quinine has been found to be the failure of the patient to take the drug. Fletcher [234] tested 223 patients in hospital daily with Mayer's reagent and found that 66, or 28 per cent, failed to show quinine in the urine although they were supposed to be on the regular treatment with quinine for malarial infection. He states that he believes that at least 25 per cent of the quinine prescribed in hospitals in India is not swallowed by the patients, and it is probably true that this condition obtains to a lesser extent in hospitals in all parts of the world. Recent authorities, therefore, advise frequent testing of the urine of patients taking quinine in order to be sure that the drug is being taken and absorbed.

*Quinine Fastness.*—The question of the occurrence of quinine-fast strains of the malaria plasmodia has attracted much attention of late years, and there are those who believe that the continued administration of quinine in insufficient doses to eradicate a malarial infection brings about a condition in the plasmodia which renders them immune to the effects of the drug. The evidence as to the existence of such "quinine-fast strains" is absolutely unreliable from a scientific standpoint, and will not bear close analytical study. It is true that malarial infection frequently relapses while the patient is taking inadequate doses of quinine, or after quinine has been discontinued, but there is not a well authenticated instance of the drug not causing the disappearance of the plasmodia from the blood if administered in proper dosage and absorbed, and it has been found that the plasmodia are just as susceptible to the drug during relapses as during the initial attack.

Fletcher [237] investigated fifty-two cases of so-called "quinine resistance" in patients suffering from malaria in India, and found that in every case the "resistance" was due either to the omission of quinine or to adulterated quinine. I have repeatedly treated "quinine resistant" patients with as good results as in other cases, provided the condition present was malarial and the patient took the drug recommended. While it would be premature to state that there is no such thing as a quinine-fast strain of the malaria plasmodium, it is true that there is no scientific evidence that such strains occur and equally true that the so-called "quinine-resistant" patients with malaria have invariably been found to be nonresistant if properly treated and the

237. Fletcher, W. (footnote 234); Bull. Int. Med. Res. F. M. S., no. 3, 1925.

quinine prescribed swallowed by the patient, provided, of course, that the symptoms present were due to malarial infection. I have never encountered a patient with a "quinine-fast" strain of the malaria plasmodia or a single initial malarial infection which failed to respond to proper treatment with quinine if the treatment was administered before fatal symptoms appeared, and my experience is the same as that of all careful students of this interesting question. Hehir,[200] as the result of his very extensive experience in India with malaria, makes a similar statement, and it is the consensus of practically all recent authorities that quinine-fast strains of the malaria plasmodia do not exist.

*Substitutes for Quinine.*—From the time of the discovery of the specific effect of quinine in the treatment of persons with malaria, efforts have been made to find a substitute that would be more pleasant to take, more efficient in destroying the plasmodia and cheaper in price. Within recent years, with the advent of chemotherapy, these efforts have been redoubled and many synthetic preparations have been advocated, from time to time, as substitutes for this valuable drug. With few exceptions, all have fallen by the wayside, but some have a limited value in treatment and can be substituted for quinine when the latter drug cannot be administered for one reason or another. The more important drugs that have been urged as substitutes will be briefly considered.

Euchinine, the ethyl carbonate of quinine, has been used as a substitute for the more common salts of quinine and has the advantage of being almost tasteless and less toxic in effect, but is much more expensive. The dose is twice that of quinine sulphate, and it is an efficient remedy for malarial infections. Methylene blue (medicinal) is effective in the treatment of tertian and quartan malaria, but less so in the estivo-autumnal infections. It is administered in doses of from 0.13 to 0.2 Gm. (2 to 3 grains) every four hours, mixed with a little powdered nutmeg, and is preferably given in capsules. It sometimes causes strangury, albuminuria and diarrhea, and the patient should be carefully watched during its administration. As the drug colors the urine blue, the patient should be warned of this fact.

The arsphenamines have been investigated by numerous observers and have been found to give good results when administered intravenously in tertian and quartan malaria, but appear to be useless in estivo-autumnal infections. As these preparations are more toxic than quinine and its salts, they should not be used except when quinine cannot be administered, and never in estivo-autumnal malaria. Stovarsol, or acetylamino-oxyphenylarsonic acid, which contains from 27.1 to 27.4 per cent of arsenic, has been recommended recently as a substitute for quinine. It may be administered by the mouth or intravenously.

This drug has been reported on by Valenti and Tomaselli,[238] Vialatte,[239] Bass,[240] Sinton,[241] Tardres,[242] Guerin, Borel and Advier [243] and others, and it has been found to be efficient as treatment in infections with *Plasmodium vivax,* but inefficient in infections with *Plasmodium malariae* and *Plasmodium falciparum.* The sodium salt, sodium stovarsol, has been recommended, and has recently been thoroughly investigated by Sinton,[244] who found it effective in infections with *Plasmodium vivax.* The drug was administered intravenously and the parasites disappeared from the peripheral blood in from five to thirty hours. Microscopic examination of the blood demonstrated that the drug caused degeneration and fragmentation of the parasites. The destruction of the plasmodia in large numbers often caused a high fever following its first injection, an effect that has also been noted after the injection of stovarsol. Raynal [245] has reported on the treatment in tertian and estivo-autumnal (subtertian) malaria with stovarsolate of quinine, a combination containing equal parts of stovarsol and quinine. He obtained good results with this combination, but the inherent weakness of stovarsol as a specific for malaria is shown by the necessity of combining it with quinine in order to achieve success in the treatment in cases of estivo-autumnal and quartan malaria.

All careful investigators of stovarsol have reported symptoms of poisoning when the drug is used in large doses or administered to patients who are unusually sensitive to arsenic. The symptoms most often noted have been abdominal discomfort and pain, severe diarrhea, neuritis, erythematous eruptions and fever, and in some patients the symptoms of poisoning have been alarming. It follows, therefore, that stovarsol should always be administered under the care of a physician, and the patient should be carefully watched during its administration for the appearance of symptoms of intolerance to arsenic.

I believe that the combined experience with these newer arsenical preparations indicates that they are a useful substitute for quinine in cases of infections with *Plasmodium vivax,* but that they are worthless in infections with *Plasmodium malariae* or *Plasmodium falciparum,* and are more toxic than any of the salts of quinine.

The most recent synthetic drug which has been recommended as a substitute for quinine is known under the trade name of "plasmochin."

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238. Valenti and Tomaselli: Gazz. d. osp. **47**:267, 1926.
239. Vialatte, C.: Bull. Soc. path. Exot. **19**:359, 1926.
240. Bass. C. C.: Southern M. J. **19**:381, 1926.
241. Sinton, J. A.: Indian J. M. Research **14**:227, 1926.
242. Tardres, J.: Arch. d. Inst. Pasteur d'Algerie, **4**:49, 1926.
243. Guerin; Borel and Advier: Bull. Soc. path. Exot. **20**:331, 1927.
244. Sinton, J. A.: Indian J. M. Research **15**:287, 1927.
245. Raynal, J.: Bull. Soc. path. Exot. **20**:665, 1927.

Plasmochin is a quinoline derivative introduced by the Germans for treatment in malaria, after observations of its effect on the plasmodia causing malaria in birds. In 1926, Roehl [246] published the results of his observations on the effect of this drug on birds with malaria, in which he stated that it is sixty times more powerful than quinine in preventing and curing malarial infection in birds. His observations were followed by those of Hegner and Manwell,[247] who found that the drug was an effective therapeutic agent for birds with malaria, but that it did not destroy all the parasites in the host and did not remove the danger of a relapse. Sioli [248] found the drug effective in the treatment of inoculation malaria in paretic persons, and urged its trial in naturally acquired infections. Mühlens [249] was the first to try the drug in natural malarial infections, and found it effective in infections caused by *Plasmodium vivax* and *Plasmodium malariae,* in which its use was followed by the prompt disappearance of the parasites from the peripheral blood. He found, however, that its action in infections caused by *Plasmodium falciparum* was much slower, although he obtained sterilization of the peripheral blood. The most important fact noted in his publication is the effect of this drug on the gametocytes, or sexual forms, of the plasmodia, causing their disappearance from the peripheral blood within from three to five days and their apparent destruction by the direct action of the preparation. Since these preliminary observations, plasmochin has been used extensively as the treatment in cases of malaria, and has been reported on by Mühlens and Fischer [250] Memmi and Schulemann,[251] Manson-Bahr,[252] Eiselsberg [253] Fletcher and Kanagarayer,[254] Fischer and Weise [255] and others. The consensus of opinion appears to be that in infections with *Plasmodium vivax* and *Plasmodium malariae,* it causes the prompt disappearance of the plasmodia from the peripheral blood and the cessation of symptoms, but that relapses are frequent even after long continued use of the remedy, and that it has less effect on the human life cycle forms (schizonts) of *Plasmodium falciparum,* although it kills the crescents, or gametocytes.

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246. Roehl, W.: Beihefte z. Arch. f. Schiffs- u. Tropen- Hyg. **30**:305, 1926.
247. Hegner and Manwell: Am. J. Trop. Med. **7**:279, 1927.
248. Sioli, F.: Beihefte z. Arch. f. Schiffs- u. Tropen- Hyg. **30**:319, 1926.
249. Mühlens, P.: Beihefte z. Arch. f. Schiffs- u. Tropen- Hyg. **30**:325, 1926.
250. Mühlens and Fischer: Beihefte z. Arch. f. Schiffs- u. Tropen- Hyg. **31**: 1, 1927.
251. Memmi and Schulemann: Riv. di Malariologica **6**:40, 1927.
252. Manson-Bahr, P.: Proc. Roy. Soc. Med. **20**:33, 1927.
253. Eiselsberg, K. P.: Wien. klin. Wchnschr. **40**:525, 1927.
254. Fletcher and Kanagarayer: Indian M. Gaz. **53**:499, 1927.
255. Fischer and Weiss: Deutsche med. Wchnschr. **53**:1380, 1927.

The makers of plasmochin have recognized the fact that the drug alone is not effective in actually curing malaria and now market it combined with quinine under the name "plasmochin compound." This compound is put up in tablets each containing 0.01 Gm. of plasmochin and 0.125 Gm. of quinine, and has been found much more efficient in the treatment of persons with malaria than plasmochin alone, and has the added advantage that gametocytes are destroyed. Plasmochin should not be given in a daily dose to exceed 0.06 Gm., the usual dosage of plasmochin compound being four tablets daily, each containing 0.01 Gm. of plasmochin, administered at regular intervals.

Early during the work on plasmochin, it was noted that symptoms of poisoning frequently occurred after its administration. Mühlens,[256] Fischer and Weise,[255] Manson-Bahr,[252] Sioli [248] and Eiselsberg [253] have all described symptoms of poisoning produced by this drug. These symptoms consist in cyanosis, with or without methemoglobinemia, albuminuria, fever, epigastric pain, vomiting and tenderness over the region of the liver, and fatal cases of poisoning have been reported. Fischer and Weiss [255] state that even with small doses methemoglobinemia occurs, as ten of thirteen patients who were taking 0.03 Gm. daily developed this condition, while seven of seventeen patients taking 0.02 Gm. daily also developed it, although the authors state that these small doses are not dangerous, and while they will not kill the schizonts, the gametocytes are destroyed. These authors recommend that small doses of plasmochin be combined with quinine for both the treatment and prophylaxis in cases of malaria. The use of the plasmochin compound in prophylaxis, owing to the danger of poisoning when administered on such a large scale, should not be attempted unless those taking it can be kept under medical supervision.

It is evident from the data that have accumulated that plasmochin alone is much less efficient in the treatment of malarial infections than quinine, and that it should be given under careful medical supervision owing to the danger of poisonous symptoms developing in those who are taking the drug. Its great advantage consists in its power of destroying the sexual forms, or gametocytes, of the plasmodia, thus rendering the infection of the mosquito impossible. Combined with quinine in small doses it would appear to be most useful in ridding the blood of gametocytes and helping to control the symptoms of infection, but it is far less efficient than quinine in the treatment of persons with malaria, so far as ultimate cure is concerned.

Among other substitutes for quinine that have been recommended are mercurochrome-220 soluble, antimony, peracrina-303, which appears to consist of yeast cells and starch, and "smalarina." All of these

256. Mühlens, P.: Deutsche med. Wchnschr. **53**:1891, 1933, 1927.

preparations have been thoroughly tested in the treatment of malarial infections and have been found worthless.

*Malaria as a Therapeutic Agent.*—While it is impossible to discuss the recent use of induced malaria as a therapeutic agent in the treatment of paretic patients in this contribution, no review of malaria would be complete without a mention of this remarkable adaptation of one infection to the treatment of another. The literature of the subject is now enormous, and to attempt to cover it would result in a review as extensive as the present one on malaria, but it may be stated that the treatment of paretic patients by the experimental production of the symptoms of malaria through the direct inoculation of blood containing the plasmodia, or through the bites of infected mosquitoes, has now become an established practice and offers the only hope for permanent benefit in the treatment of these unfortunate persons. In a recent compilation of the data recorded in the literature on 3,193 patients with paresis treated by the induction of malaria, Dante [257] states that there were complete remissions in 30 per cent, marked but incomplete remissions in 20 per cent, slight improvement in 20 per cent, and no change in 20 per cent. The percentage of deaths attributable to the malarial infection was 10 per cent. Wagner-Jauregg,[258] the originator of this method of treatment, has recently summed up his experience with it, and the reader is referred to this valuable summary of the methods of conducting the treatment and the results which have been obtained. He states that deaths from malaria have almost ceased, and that in 2,000 cases the malarial infection has invariably been cured by 5 Gm. of quinine administered within one week.

257. Dante, F.: Ann. di Med. Nav. e Colon, **32**:149, 1926.

258. Wagner-Jauregg, J.: Wien. klin. Wchnschr. **60**:1121, 1927.

# Notes and News

**Fund for the Study of Leukemia.**—It is reported that the University of Pennsylvania has received $100,000 to be devoted to the study of leukemia and allied conditions. This study will be carried out under the direction of Eugene L. Opie.

**General Statement of Policy from the Committee on Research in Syphilis.**—The committee desires to aid by grants the development of individual and of cooperative investigation by qualified scientists and agencies for a well defined plan of attack on the problems of syphilis. These grants will be given to aid in the more vigorous prosecution and expansion of existing researches, and the development of additional studies in laboratory, clinical and other problems.

Grants will be made in quarterly allotments for a period of one year, subject to consideration for renewal. The approval of president and trustees is necessary for an institutional grant. Application forms may be obtained on request, from Dr. John H. Stokes, chairman, scientific committee, 3800 Chestnut Street, Philadelphia. A personal interview may be necessary, but only on request of the committee.

While a definite program of attack on syphilis is being formulated and major effort is being directed toward its realization, the committee is desirous of receiving and evaluating current ideas as to the points on which syphilis as a present-day medical and public health problem is vulnerable to attack. Such proposals should be as concrete as possible. The committee will welcome concrete problems in syphilis, and the names of available investigators, and promising investigations, here and abroad. It is desirable that such proposals be accompanied by definite statement as to methods and objectives. The policy of the committee is to aid research in syphilis by providing additional personnel and equipment rather than by augmenting existing salaries. In specific cases, however, decisions will be made on the individual merit of the problem or plan and the capacity of the investigator or clinic.

Those making applications for grants will be asked to state clearly the nature of the problem to be attacked, the information sought and the methods it is proposed to employ; describe the quarters, material, equipment, personnel and budget now at hand for the conduct of such investigation; describe the additional equipment required; describe the additional personnel required; present a general estimate of the total cost and duration of the research; present an annual budget in quarterly estimates with as definite allotments and as small a miscellaneous item as possible; and submit for examination necessary credentials evidencing general fitness of the personnel or organization for the work planned, and of ability to carry through an undertaking once begun.

In general, grants will not be made for permanent plant or architectural alterations. Equipment purchased for investigations remains the property of the committee and is to be regarded as a loan except as used up in the course of the work. Repairs in general and unavoidable breakage should be budgeted.

The individual autonomy of the investigator will be painstakingly considered and preserved. The committee reserves the privilege of conference with the investigator; and will require informal reports by letter at quarterly intervals, on the progress of investigations. Conferences and discussions between groups acting as jurors will be utilized when advisable.

Publication of results is to be with the approval of the scientific committee.

# Abstracts from Current Literature

## Pathologic Physiology

THE INFREQUENCY OF SEVERE RICKETS IN NEW ORLEANS AND VICINITY. C. T. WILLIAMS, Am. J. Dis. Child. **35**:590, 1928.

Severe rickets is extremely rare in New Orleans and vicinity, but a mild form of the disease prevails in about 45 per cent of children, aged 3 years or less. A group of children was studied as to the incidence of rickets and the possible relationship between the disease and certain factors of locality, meteorologic conditions, diet and home environment. The annual phosphorus level in the blood of children in this vicinity was considerably higher than that reported for children in New York. There was a direct relationship between the content of inorganic phosphorus in the blood and the percentage of possible sunshine for each month. Green vegetables in the diet apparently had some protective influence against the disease, but the factor considered most important for the occurrence of the mild form of rickets in this region was the geographic location.

PEARL ZEEK.

VITAMIN REQUIREMENTS OF NURSING YOUNG. BARNETT SURE and S. J. SHILLING, Am. J. Dis. Child. **35**:811, 1928.

Observations were made on the pathologic lesions encountered in the polyneuritic state of the nursing young of the albino rat that had received inadequate amounts of vitamin B. Hemorrhages were found in certain of the viscera and in the osteogenic tissues. The animals usually died with their stomachs well distended with curd. Diets which produced beriberi and polyneuritis in the nursing young were found entirely satisfactory for mature animals and for the growth of non-lactating subjects.

PEARL ZEEK.

COUGH—ITS ACTION ON MATERIAL IN THE TRACHEOBRONCHIAL TRACT. E. ARCHIBALD and A. L. BROWN, Arch. of Surg. **16**:322, 1928.

The authors injected iodized oil and iodized oil mixed with sputum into the trachea of cats, and found that in the animals that coughed, the oil was carried deeper into the lung tissue and remained there much longer than in the animals that did not cough. The forced expiratory effort of coughing is immediately preceded or followed by a markedly increased inspiration. This inspiratory effort spreads material in the bronchi deeper into the pulmonary tree, instead of expelling it.

N. ENZER.

RELATION OF THE PARATHYROIDS TO THE HEALING OF A FRACTURE AS CONTROLLED BY THE ROENTGEN RAYS. D. E. ROSS, Arch. Surg. **16**:922, 1928.

Ross studied the effects of the removal of parathyroid glands on the healing of fractures in cats. In a certain series he removed two of the parathyroid glands and fractured one of the long bones. Determinations of the blood calcium and blood phosphorus were made at frequent intervals. In another series he removed three of the parathyroid glands. When only two of the glands were removed, delay in the union of the fractures did not result, but when three of the glands were removed, bony union did not appear, according to the roentgenograms, until the fifth or sixth week. In these animals the blood calcium was reduced, and the deposition of callus was coincident with the return to normal of the blood calcium. The blood phosphorus level was not affected by the parathyroidectomy.

N. ENZER.

EFFECT OF HIGH PROTEIN DIETS ON THE REMAINING KIDNEY OF RATS. H. JACKSON and O. J. MOORE, J. Clin. Invest. **5**:415, 1928.

After the removal of one kidney, eighteen rats of varying ages were placed on an adequate diet containing 70 per cent casein. Urinalyses and pathologic studies of the kidneys were made. Lesions of the kidney of greater or lesser extent were found in some rats but not in others. Older animals appeared more liable to renal damage, but it is suggested that individual susceptibility is a factor.

H. R. FISHBACK.

THE TOXIC EFFECTS OF AMINES. A. R. JOHNSTON, J. Infect. Dis. **42**:473, 1928.

Death from the amines used in these experiments is the result of respiratory failure.

No relationship exists between the number of $NH_2$ groups in the molecule and its toxicity, including its edema-producing qualities. Pyridine and quinoline in which the nitrogen is bound with the ring produce general anesthesia. Cadaverine and putrescine are not toxic, and fuchsin only slightly so as compared to the markedly toxic paraphenylenediamine.

Paraphenylenediamine on living rabbits manifests its action first peripherally (loss of locomotion), second by early acceleration of respiration, third by salivation, sonorous and labored breathing, edema of the tongue, nose, face, neck and salivary glands, or if sufficient amount is given, clonic seizures terminate the life of the animal before the edema stage is reached. It produces marked rigor in frogs. The hydrochloride is less toxic and has less edema-producing qualities than the base. Calcium chloride given with the compound partially antagonizes its action. Tolerance to it is not obtained.

Various amines have predilection for different tissues of the same animal. Paraphenylenediamine has predilection for the same tissues removed from the body as in the living animal. Amines affect the action of the exposed intact frog heart but only in concentrations above those tolerated in the living animal.

Basic amines cause an increase in both muscle rigor and the swelling of muscles; both the rigor and swelling are greater than is produced by acids at any concentration. The salts of the basic amines diminish both the rigor and the swelling of muscles. Increased swelling of muscles may occur with relaxation.

AUTHOR'S SUMMARY.

THE TWO PRODUCTS OF THYROID ACTIVITY. G. S. WILLIAMSON, I. H. PEARSE and H. M. CUNNINGTON, J. Path. and Bact. **31**:255, 1928.

The authors have investigated the possibility of a duality of function in the physiologic cycle of the thyroid gland. Chemical analysis of hyperplastic or secreting tissue showed the presence of small quantities of iodine in only 6 per cent of 200 samples analyzed, while 100 samples of colloid tissue all yielded iodine. Thyroxin fed to tadpoles causes early metamorphosis and checks growth. Making use of this biologic test, it was found that the effect of thyroxin is exclusively the property of iodocolloid; that all of the iodine in the thyroid is not biologically active, and that secretion tissue (hyperplastic) does not induce premature metamorphosis in tadpoles and presumably does not contain thyroxin. E. M. HALL.

ACIDITY OF GASTRIC SECRETION AND URINE. H. SCHULTEN, München. med. Wchnschr. **75**:898, 1928.

The normal urine $p_H$ curve is definitely associated with the secretion of hydrochloric acid in the stomach. The secretion of hydrochloric acid, however, is not the only cause of such variations. Each formation of hydrochloric acid by the stomach is not followed invariably by an alkalinity in the urine.

AUTHOR'S SUMMARY.

TISSUE RESPIRATION OF NORMAL AND TUBERCULOUS ANIMALS. W. KELLER, Ztschr. f. d. ges. exper. Med. **58**:117, 1927.

This article contains a detailed discussion of the methods essentially that of Warburg for measuring tissue respiration. Sections of the surviving kidney, liver, and spleen of normal and of tuberculous guinea-pigs were studied. The excised tissues were treated with tuberculin and various extracts of tubercle bacilli. No difference in respiration or glycolysis of tissues from normal or infected animals was found, unless actual tuberculous foci were present in the tissues examined. The respiration of tissues containing tuberculous foci was about 50 per cent higher in value than that of normal (nontuberculous) tissue. Treatment of tissue from normal and from tuberculous animals with tuberculin or extracts of tubercle bacilli did not bring out any specific differences that could be determined by measuring respiration or glycolysis.

B. LUCKÉ.

ORIGIN OF EXTRASYSTOLES. D. SCHERF, Ztschr. f. d. ges. exper. Med. **58**:221, 1927.

If one initiates a series of extrasystoles through stimulation of a localized spot on the surface of the ventricle of a heart of a dog that has been treated with barium, this series will last longer if the primary or secondary centers have been inhibited by simultaneous vagus stimulation than if the vagus is not stimulated.

The slow disappearance of the series always occurs in a similar manner, so that the author assumes that conduction disturbances from the ectopic focus to the ventricular muscle play only a subordinate rôle and that it is much more likely that the ectopic focus gradually functions more and more slowly until it finally stops.

After disappearance of the extrasystoles, vagus stimulation can make them reappear. They reappear at the same instant at which they would have occurred if they had not disappeared—a further proof to the author of the existence of a protective blocking.

At a certain stage of the barium poisoning extrasystoles appear regularly after vagus stimulation, even if the focal irritation is not applied. These do not show any protective blocking, but are the expression of an increased degree of automatism.

At a certain phase of the barium poison, accelerator stimulation will also produce a long series of extrasystoles.

Confirmation of the origin of the extrasystoles from the ectopic focus is afforded by the observation that warming the focus increases the rate of the extrasystoles.

In hearts treated with small doses of barium, transient extrasystoles were obtained whenever warmth was applied. The evidence seemed to show that these were due to warming the Purkinje network and not the main mass of muscle.

Extrasystoles produced by strophanthin were not influenced by warmth.

Extrasystoles produced by warming one ventricle were said to be transmitted to the other by the ordinary musculature and the Purkinje fibers and not by a branch of His' bundle.

Electricially induced auricular extrasystoles never bring about a typical series; stimuli at or near the sinus node, however, produce them with greater frequency, which leads the author to assume that persistence of extrasystoles is associated with involvement of specific tissue.

B. LUCKÉ.

"BLOCKAGE" OF THE RETICULO-ENDOTHELIAL SYSTEM. S. LEITES and A. RIABOW, Ztschr. f. d. ges. exper. Med. **58**:314, 1927.

The ability of the reticulo-endothelial system to store electronegative colloids introduced into the blood was measured by the rapidity of elimination from the blood of the substances introduced. Colloidal saccharated ferric oxide was selected as the material to be injected, because it is known to be nontoxic, it is stored almost exclusively in the reticulo-endothelial system (in the restricted sense) and

determinations of iron can readily be made in the blood. A standard method of preparation of the material and a constant technic of injection is described. The solution was injected into the saphenous veins of dogs in amounts of from 5 to 20 cc. The amount of iron in the blood was determined before and at certain intervals after injection after the method of Pincussen ("Mikromethodik," third edition). The number of erythrocytes was determined in the usual manner. In normal dogs the intravenous injection of true solutions of iron (iron sulphate, iron glycerophosphate) brings about an increase of iron in the blood (60 to 120 per cent) within five minutes after injection. Colloidal iron does not bring about any increase. Histologic studies showed that the injected colloidal iron is stored within five minutes by the reticulo-endothelial cells. There is no change in the number of erythrocytes. If the reticulo-endothelial system is blocked by repeated injection of "solargentum" or colloidal iron, the ability of the reticulo-endothelial system to store injected colloidal iron varies in one of two directions: moderate blockage leads to a decreased storage, prolonged blockage to an increased storage. In other words, in moderate blockage there is an increase of iron in the blood (due to nonstorage), but such an increase does not occur if blockage has been prolonged. The latter phenomenon is due to proliferation of the reticulo-endothelial cells. The reaction of the reticulo-endothelial system to blockage is therefore biphasic, and depends on the duration and intensity of the blockage. After splenectomy, the duration of iron from the blood by storage in the reticulo-endothelial cells is somewhat delayed, but the loss of reticulo-endothelial cells through splenectomy is soon compensated for by hyperplasia of the reticulo-endothelial system in the liver and bone marrow.

B. LUCKÉ.

CONCERNING THE INFLUENCE OF THE SPLEEN ON IRON METABOLISM. E. LAUDA and E. HAAM, Ztschr. f. d. ges. exper. Med. **58**:322, 1927.

This is a continuation of Lauda's work on the effect of splenectomy in rats in which he attributes the severe anemia and frequent death to the lighting up of an infectious anemia in rats (Wien. Arch. f. inn. Med. **11**:294, 1925, and **13**:190, 1926; also Ztschr. f. d. ges. exper. Med. **55**:505, 1927).

Noting Lepehne's observation of the great accumulation of iron in the Kupffer cells of the liver and the observation of Ascher and Tominaga that iron could be demonstrated chemically in larger amounts in the liver after splenectomy, Lauda has investigated the effect of infectious anemia of rats in producing similar conditions in the absence of splenectomy. The iron content of the liver was noted in three series of rats; twelve normal rats, twelve splenectomized rats that were also anemic, and twelve splenectomized rats that were not anemic. From the protocols, which are not suitable for abstracting, it became apparent that the iron content of the liver in the normal and nonanemic splenctomized rats was similar, whereas the anemic splenectomized rats showed a much higher iron content in the liver. The author does not deny a compensatory action of the liver and other organs of the hemolyticopoietic system after splenectomy, but maintains that in this particular species the infectious anemia overshadows splenectomy.

B. LUCKÉ.

CORRELATION BETWEEN OXYGEN CAPACITY AND TOTAL AMOUNT OF HEMOGLOBIN (CONTRIBUTION TO THE PATHOGENESIS OF POLYCYTHEMIA). E. BAUER and K. LAWROWSKI, Ztschr. f. d. ges. exper. Med. **58**:586, 1927.

It is a known fact that cases of polycythemia often exhibit symptoms of oxygen hunger, in spite of the marked increase in total hemoglobin; these symptoms are favorably influenced by inhalation of oxygen. The object of this study was to determine whether the oxygen capacity of hemoglobin from cases of polycythemia was diminished, and whether the increase of total hemoglobin in the disease represents a compensatory reaction to diminished oxygen capacity of hemoglobin. One hundred and three determinations on ninety-five persons were made; among these there were twelve healthy persons and two patients with polycythemia. It

was found that, in general, increase of the total hemoglobin content of the body is associated with decrease in oxygen capacity and, conversely, with a diminished total hemoglobin content of the body there is an increased oxygen capacity. This correlation was found to exist in both healthy and diseased persons, and in the two cases of polycythemia. Polycythemia is therefore regarded as an extreme instance of a compensatory relation between oxygen capacity and hemoglobin content of blood.

B. LUCKÉ.

VASCULAR REACTION OF ISOLATED ORGANS OF ANAPHYLACTIC ANIMALS. S. GENES and Z. DINERSTEIN, Ztschr. f. d. ges. exper. Med. **58**:629, 1927.

Isolated organs (ear, spleen, liver, kidneys, lungs) of normal rabbits and of rabbits previously sensitized with horseserum were perfused with Ringer-Locke solution containing horse serum in various dilutions. Contraction of vessels was determined by counting the number of drops per minute of the perfusate. Perfusion of normal ears with serum diluted from 1:100 to 1:1,000 did not cause any definite vascular response; with dilution of 1:50, it caused moderate contraction. In the ears of sensitized animals perfusion with serum generally produced a marked vascular contraction; with dilutions of 1:50 it gave rise to extreme contraction (spasm). Perfusion of the internal organs of normal and of sensitized animals brought out little difference; at most, there was a slight vascular contraction in some.

B. LUCKÉ.

INVESTIGATIONS ON THE HISTOLOGIC CHANGES IN THE SKIN OF RATS ON VARIOUS DIETS. KAI PORTMAN, Acta path. et microbiol. Scandinav. **4**:341, 1927.

In rats on diet without vitamin A and B, as well as in rats partially starved, there occurred atrophy of the skin with loss of hair.

## Pathologic Anatomy

BLOOD VESSELS IN THE VALVES OF NORMAL HUMAN HEARTS. SAUL A. RITTER, LOUIS GROSS and M. A. KUGEL, Am. Heart J. **3**:433, 1928.

The hearts of 700 human beings have been studied with reference to the existence of blood vessels in the valves. Of these, fourteen normal hearts have been described which present blood vessels in some of the valves. A thorough clinical and pathologic examination has failed to reveal that these vessels owe their origin to inflammation. Additional reasons are given which render it highly improbable that they owe their origin to inflammation, or that they are other than embryonic vestiges.

AUTHORS' SUMMARY.

A YOUNG HUMAN OVUM IN SITU. J. P. Greenhill, Am. J. Anat. **40**:315, 1927.

A human ovum, obtained in an aborted decidual cast, is described. Maximum measurements are: the ovum, including the decidua capsularis, 8.37 by 6.44 by 2.67 mm.; the implantation cavity, 6.23 by 5.79 by 2.5 mm.; the ovum with the definitive villi, 4.72 by 5.41 by 2.23 mm.; the ovum, exclusive of villi, 4.08 by 4.21 by 1.58 mm.; the embryonic disk (estimated), 0.15 by 0.13 mm., and the yolk sac, 0.64 by 0.53 by 0.43 mm.

The outstanding feature of this ovum is the unusual amount of invasion of the decidua by plasmodium. There is abundant trophoblast, especially plasmoditrophoblast, in the intervellous space. The chorionic vesicle has numerous villi, some of which have begun to branch. Anlages of blood vessels are found in the mesoderm of the chorionic vesicle, the villi and the yolk sac. Preserved sections of the embryo show a small amniotic vesicle and an embryonic shield lying on a large yolk sac. In the decidua capsularis is an operculum deciduae (Teacher, 1925) con-

sisting of fetal ectoderm, and it is attached to the chorin laeve by a strand of plasmodium. The decidual reaction is more marked in the stroma cells than in the glands. The development of this ovum tallies with the conception of ova as having a maximum age of 19 days.

AUTHOR'S SUMMARY.

THE CLAVICULAR EPIPHYSES. T. WINGATE TODD and JOSEPH D'ERRICO, JR., Am. J. Anat. **41**:25, 1928.

1. Epiphyses may never ossify or may ossify only in part. Union of an ossified epiphysis is comparable in date and in nature with a glazing of the naked shaft end whereby its typical coral-like texture is forever obliterated. Both phenomena are types of expression of the termination of growth, but need not necessarily follow this termination.

2. The sternal epiphysis does not always completely ossify; the lateral epiphysis may not ossify at all.

3. The sternal epiphysis unites in the twenty-fifth year; the lateral epiphysis, in the twentieth year. If either end of the clavicle is naked, it glazes at the dates mentioned. Anomalies of retardation or acceleration of not more than two years do occur.

4. Sex and stock (white or American negro) do not appear to have any power of modification of these dates.

AUTHORS' SUMMARY.

HISTOLOGIC STUDY OF THE THYROID OF THE GUINEA-PIG IN EXPERIMENTAL SCURVY. K. D. HARRIS and E. A. SMITH, Am. J. Physiol. **84**:599, 1928.

Advanced scurvy in the guinea-pig was accompanied by a marked increase in the number of the interfollicular (intra?) cells, increased vacuolization of the colloid and a lengthening of the lining cells of the follicle. Similar changes were not observed following acute starvation.

H. E. EGGERS.

STUDIES IN EXHAUSTION DUE TO LACK OF SLEEP. T. H. BAST, J. S. SUPERNAW, B. LIEBERMAN and J. MUNRO, Am. J. Physiol. **85**:135, 1928.

In the thyroid glands of exhausted rabbits the number of mitochrondria was greatly decreased. The nuclei were swollen, and the decreased chromatin was clustered along the nuclear membrane. In the suprarenal gland exact observation was difficult because of the varying pictures of supposedly normal animals. Certain observed changes were regarded as significant. In most of the suprarenal glands studied there was marked vacuolization of the cortical cells, eosinic staining of many of the nuclei and pyknosis of others. A number of the rabbits showed nests of eosinophils in the cortical sinuses. In many there was marked congestion of the medullary sinuses with either colloidal material or cellular débris. Where cortex and medulla join, most of the cells were large and embryonic in appearance, with degenerating cells between them.

H. E. EGGERS.

THE RETICULUM OF THE HUMAN SPLEEN. NATHAN CHANDLER FOOT, Anat. Record **36**:79, 1927.

In a morphologic study of a series of spleens removed at necropsy and studied in sections of fixed tissue, the observations may be summed up as follows: 1. The splenic reticulum is best demonstrated by silver impregnations of paraffin sections; perfusing the organ somewhat interferes with successful impregnation; hence, more than one method should be employed before definite conclusions concerning the histology of the reticular tissue are drawn. 2. The reticulum is everywhere continuous, but its arrangement differs with its situation: In the capsule and trabeculae it is coarse and straight; around the arterioles it is complex and weblike or feltlike, as it is in the ellipsoids; in the splenic pulp and corpuscles it is indistin-

guishable from lymphoid reticulum in general, and where it surrounds the intralobular veins, their radicles and the venous sinuses, it encloses them in distinctive annular fibrils. These do not form unbroken rings, but anastomose sparingly, and are frequently interrupted in their continuity and course. 3. There are no coarse, parallel fibers of reticulum in the walls of the veins or venous sinuses; apparently the longitudinal fibers in the pulp, or the nonreticular fibers that lie on the endothelial cells, have been mistaken for part of the venous reticulum.

AUTHOR'S SUMMARY.

ON THE ENDOTHELIUM OF THE VENOUS SINUSES OF THE HUMAN SPLEEN. NATHAN CHANDLER FOOT, Anat. Record **36**:91, 1927.

The peculiarities of the venous endothelium in the spleen are set forth and discussed from the standpoint of the current conceptions: the discrepancies of the prevailing ideas concerning its structure are pointed out.

A report of the results of a morphologic study of the venous endothelium in this organ is then made. The methods used for the investigation are described; the stains, silver impregnations and methods of examination and fixation are listed. By means of silver tannate and a combined silver-ammonium-carbonate impregnation with Mallory's phosphotungstic acid hematoxylin, preparations have been obtained that throw a great deal of light on the true make-up of this specialized endothelium and its appendages. It has light on the true make-up of this specialized endothelium and its appendages. It has been studied, in this investigation, in its fresh state, in sections of fixed material and in perfused, still viable spleens.

Peculiar bands or striae, corresponding to those mentioned by Szymonowicz, are described as lying on the outer surface of the endothelial cells; these bands are discussed at some length. No definite conclusion is drawn as to their significance, although conjectures are made as to whether or not they represent contractile organs or mere localized thickenings on the surface of the cells. The latter hypothesis is favored, as evidence of their contractile nature is still lacking.

AUTHOR'S SUMMARY.

A NOTE ON A RARE CONGENITAL PELVIC KIDNEY. M. B. GILMAN, Anat. Record **36**:149, 1927.

A normally sized, functioning kidney in the midpelvic position was composed of three lobes, each with an artery; two lobes were supplied from the crotch of the aortic bifurcation, the other from the left internal iliac artery. The venous return was into the vena cava and left internal iliac vein. The ureter was short, 4 inches (10 cm.) in length, but coursed normally in the pelvis. Each suprarenal gland occupied the normal lumbar position, and in the absence of a normally placed left renal vein, the spermatic vein entered the left suprarenal vein. The available hospital records showed the condition had never caused trouble.

Since this segmental excretory blastema lies in close proximity to the umbilical artery and the aorta, which latter vessel is segmented in the embryo, the arterial supply is established to this segmental kidney. Biochemotaxis then attracts the ureter bud. The permanent functioning kidney is thus established. The venous return is established in much the same way as the arterial supply. Classification of such abnormalities and a short review of the literature are given.

AUTHOR'S SUMMARY.

SOME INTERESTING ANOMALIES OF THE HUMAN BODY. K. S. CHOUKE, Anat. Record **36**:389, 1927.

Four anomalies are described: (1) lateral internal mammary artery, a rare branch of the internal mammary and of some clinical importance in paracentesis thoracis; (2) femoral nerve and iliacus muscle—the femoral nerve was bifurcated in a part of its course and a portion of the iliacus muscle passed through this gap in the nerve; this may have a bearing on the pain in the region of distribution of

the femoral nerve on contraction of the iliacus muscle; (3) musculus adductor hallucis accessorius—an extra muscle originating from the medial border of tibia and medial surface of calcaneus and inserting with the oblique head of the adductor hallucis muscle; and (4) carotid arteries—an unusual anastomosis between internal and external carotid arteries is explainable as persisting ventral part of the second aortic arch.

AUTHOR'S SUMMARY.

THE HEART OF A CHILD, AGED 22 MONTHS, PRESENTING AN ANOMALOUS VEIN FROM THE PULMONARY AURICLE TO THE RIGHT INTERNAL JUGULAR VEIN, TRANSPOSITION OF THE GREAT VESSELS AND LEFT SUPERIOR VENA CAVA. H. A. HARRIS, S. H. GRAY and CAROLINE WHITNEY, Anat. Record **37**:31, 1927.

The embryologic basis of the anomalies is discussed, particularly regarding the jugulopulmonary vein. This rare vessel is shown to be a persistence of the anastomotic channels between the intestinal plexus and the anterior cardinal vein on the one hand and the intestinal plexus and the veins of the lung bud on the other. The problem of the reversal of blood flow through the persistent left superior vena cava, through the patent ductus and through the other arteriovenous shunts is considered.

A possible mechanism is suggested for the erythema and increased blood volume which is seen in certain cases of congenital heart disease. The general incompatibility between the clinical forecast and the actual postmortem observations is stressed.

AUTHORS' SUMMARY.

DESCRIPTION OF A YOUNG HUMAN ANENCEPHALIC AND AMYELIC EMBRYO. ERNEST DE VRIES, Anat. Record **37**:293, 1927.

A human embryo, measuring 23 mm. is described, with anencephaly and amyelia. The hemisphere and thalamus are present; the latter has an opening leading into its ventricle and also perforating the membrane which covers the brain. Of the cranial nerves, the olfactory and optic nerves are present, and the sensory nerves from their ganglions or including a little stretch of the roots. The motor nerves are also present, without any central connections. In the cervicothoracic part a groove takes the place of the spinal cord and vertebral arches. This groove is covered by normal skin, below which lie the spinal ganglions. More caudally, the groove closes dorsally; then it is not lined with skin, but with endothelium, and represents the meningeal sac (no differentiation as yet exists between pia and dura). In this sac a few remnants of spinal cord are found. The rate of development of the embryo must have been much slower than normally, the ovum probably was 12 weeks old. The abnormal dorsal flexion of the head is secondary to the absence of nerve tissue.

An attempt is not made to find the cause for this deformity, but five other cases from the literature are briefly discussed, and those points summed up which need to be cleared up before an explanation of these deformities can be given.

AUTHOR'S SUMMARY.

OBSERVATIONS ON THE LYMPHATIC CONNECTIONS OF THE THYROID GLAND IN MAN. H. R. MAHORNER, H. D. CAYLOR, C. F. SCHLOTTHAUER and J. DE J. PEMBERTON, Anat. Record **37**:341, 1927.

By direct injection of diluted India ink into the substance of the thyroid gland, it was demonstrated that the lymph vessels leave the gland mainly at the superior poles, at the inferior poles and at the middle of each lateral lobe. These vessels generally pass to lymph nodes of the deep cervical group, but in three of twenty preparations a lymphatic vessel from the right lobe of the thyroid emptied directly into the right subclavian vein without the interposition of any lymph nodes.

AUTHORS' SUMMARY.

SINUS PERICRANII (REDUCIBLE BLOOD TUMOR OF THE CRANIUM). E. V. HAHN, Arch. Surg. **16**:31, 1928.

Sinus pericranii is the best term to designate a blood cyst of the pericranium communicating with an intracranial blood sinus by one or more abnormal foramens in the skull. Clinically, it presents a soft, compressible, fluctuant tumor, which increases in size whenever the intracranial tension is increased, such as by posture, or compression of the jugular veins. The cases reported in the literature so far have lacked many details. Trauma appears to play some part in its formation, as it did in the case reported by Hahn. At operation there were numerous radiating blood vessels between the tumor and the surrounding tissue. The microscopic picture was that of a hemangioma in which communication could be traced between the small vessels of the septums and the cavernous spaces. This, the author believes, explains the lesions as being due to abnormal arteriovenous communications, and reinforces Mont Reid's opinion that all simple hemangiomas arise in this manner.

N. ENZER.

PATHOGENSIS OF GOITER CONSIDERED AS ONE CONTINUOUS DISEASE PROCESS. A. E. HERTZLER, Arch. Surg. **16**:61, 1928.

The interpretation of the various forms of goiter and hyperthyroidism as stages in a continuous disease process is suggested by clinical and anatomic studies. In long standing goiters, the first stage is generally an accumulation of colloid. The gland is soft and elastic. New gland formation is indicated by the presence of small alveoli lined by columnar cells. These Hertzler asserts arise from the interstitial cells. From then on the process is one of involution and regression—the end-result depending on which force predominates. Thus, the so-called adenoma is really a nodular colloid goiter, the toxic adenoma is such a goiter showing increasingly active proliferation of newly formed acini. In acute hyperthyroidism or in exophthalmic goiter, in which the presence of an enlarged thyroid was not previously noted, the hyperplasia is much more diffuse and uniform.

N. ENZER.

RELATION OF HYPERTHYROIDISM TO BENIGN TUMORS OF THE THYROID GLAND. W. F. RIENHAFF and D. LEWIS, Arch. Surg. **16**:79, 1928.

This article is a further elaboration of previous publications, and further substantiates the theory that so-called adenomas of the thyroid are not true neoplasms; hence, the terms toxic adenoma or hyperfunctioning, adenomatous goiter are misleading and incorrect. The authors have previously shown that in seven cases of hyperplasia of the thyroid studied before and after the administration of iodine, the iodine involution brings about a reversion to the colloid state, and they now show that in many instances, about 92 per cent of their cases, this colloid involution progresses to the point where a nodular thyroid results. These nodules are not neoplasms, but rather involutionary bodies, and are the regressive results of previous hypertrophy and hyperplasia. In any nodular goiter the number and size of these nodules are dependent on the chronicity, the severity, and the number of remissions in the course of the disease. Moreover, they demonstrate that the hyperplasia and hypertrophy may be confined to certain areas in the thyroid gland, so that after repeated cycles of activity and remission localized and encapsulated nodules develop. The capsules result from the pressure atrophy on the surrounding more or less inactive gland. The clinical picture of these cases of nodular goiter is merely an attenuated one of that seen in the typical exophthalmic goiter. The histologic picture of these nodules does not conform with the recognized tumors of the thyroid, but rather retains a microscopic appearance of hypertrophy and hyperplasia. In their series the authors were unable to show that the presence of a benign neoplasm was the cause of hyperactivity of the gland or of toxic manifestations, for in those cases diffuse involvement around and away from the tumor was demonstrated. Furthermore, there is no proof that benign tumors of the thyroid can

give rise to toxic manifestations by virtue of a peculiar secretion. They recommend that the following terminology be used—nodular goiter with or without hyperthyroidism, and diffuse goiter with or without hyperthyroidism.

N. ENZER.

KOHLER'S DISEASE. L. W. ELY, Arch. Surg. **16**:560, 1928.

Ely reports six cases of Kohler's disease of the tarsal navidular bone, and shows that only one of these presented some difficulty in diagnosis. In all the others the roentgenogram was typical. In the roentgenogram the bone was seen to be irregular and much denser. The clinical history revealed usually a mild injury or a low grade of infection, and also close scrutiny often revealed abnormalities in other bones of the foot. Five of the six patients recovered promptly, with little if any treatment. The condition may exist without symptoms. The author believes it is a developmental anomaly.

N. ENZER.

TORSION OF THE OMENTUM WITHOUT HERNIA. GOLDER L. MCWHORTER, Arch. Surg. **16**:569, 1928.

Torsion of the omentum may occur in the absence of hernia or any pathologic condition and without previous abdominal symptoms. There is almost always evidence of a preexisting pedicle, which may be congenital in origin. Obesity of the omentum is present in the majority of cases, and it is probably a frequent predisposing factor both in the formation of a pedicle and in the torsion. Hyperemia may be the usual exciting factor in torsion, although trauma or unusual physical exertion may initiate it.

AUTHOR'S SUMMARY.

OSTEOCHONDRITIS DISSECANS. S. BURT WOLBACK and NATHANIEL ALLISON, Arch. Surg. **16**:1176, 1928.

It is suggested that the separation of the fragments in the case examined resulted from mechanical pressure on a portion of the articular cartilage and cancellous bone bridging a so-called cyst. No explanation is at hand of the origin of such cysts, which are regions in which bone trabeculae have disappeared.

MIXED TUMORS OF THE THYROID GLAND. ARTHUR E. HERTZLER, Arch. Surg. **16**:1187, 1928.

Mixed tumors are true tumors of the thyroid gland. They are comprised of acini more or less characteristic of immature thyroid tissue. The fibrous tissue is prone to have a characteristic keloid-like proliferation and mucoid degeneration. The acini may develop colloid and later undergo the changes of an old colloid goiter, leading to toxicity. The toxicity never reaches the degree of a true exophthalmic goiter. The tumors may menace the life of the patient by a hemorrhage into their substance. They may undergo active acinal proliferation leading to a malignant growth.

AUTHOR'S SUMMARY.

INTRABRONCHIAL POLYPOID ADENOMA. DAVID REISNER, Arch. Surg. **16**:1201, 1928.

A case is described of polypoid adenoma of a large bronchus developed from the excretory duct of mucous glands, with bronchiectases.

THE ELECTROPHORESIS OF THE BLOOD PLATELETS OF THE HORSE WITH REFERENCE TO THEIR ORIGIN AND TO THROMBUS FORMATION. HAROLD A. ABRAMSON, J. Exper. Med. **47**:677, 1928.

The cataphoretic velocity of blood platelets (horse in plasma has been found to be between 0.40 and 0.51 microns per section per volt per centimeter. The mean velocity obtained from five horses is 0.45 microns per second per volt per centi-

meter. The cataphoretic velocity of polymorphonuclear leukocytes in similar specimens is practically identical with that of the platelets. This is noteworthy because of the fact that lymphocytes and red cells have different speeds. With spontaneous agglutination of platelets, white cells and red cells, there is not any change in the cataphoretic velocity incidental to aggregation. The possible surface composition of platelets and white cells is briefly discussed. The bearing of these observations on the origin of blood platelets and the mechanism of thrombus formation is demonstrated.

AUTHOR'S SUMMARY.

THE RELATIVE REACTION WITHIN LIVING MAMMALIAN TISSUES. ROBERT ELMAN, D. R. DRURY and PHILIP D. MCMASTER, J. Exper. Med. **47**:777 and 797, 1928.

The tubules of the mammalian kidney, vitally stained with erythrolitmin, show a significant color pattern, the cells of certain regions appearing bright blue and others brilliant red. The dye is segregated within the cytoplasm, staining fine granules diffusely. Under normal circumstances of renal function, erythrolitmin is stored in the lining cells of the glomerulus and the epithelial cells of the proximal convoluted tubules in the blue, alkaline form. In the cells of the final portion of the distal convoluted tubules the dye is red. Between these regions a narrow transitional zone can be found, at times above, and at times below, the loop of Henle, in which the erythrolitmin-stained granules in the tubule cells are violet, or red and blue within the same cell. Alterations in the relative reaction of certain regions of the tubules, as disclosed by the color of the dye within the cells, can be induced by means that alter the reaction of the urine. When acid urine is being secreted, there is a change from relative alkalinity to acidity high up in the tubular canal, in the proximal convoluted tubules. During the secretion of markedly alkaline urine the intracellular granules appear blue most of the way down the tubular canal, even to the first portion of the distal convoluted tubules. When the urine is neutral the cells above the descending limb of the loop of Henle are alkaline to erythrolitmin, and those below this point appear acid. The granules within the endothelial cells of the glomerulus and the epithelial cells of the first portion of the proximal convoluted tubules are always alkaline to erythrolitmin; while those within the cells of the final portion of the distal convoluted tubules are always acid regardless of the reaction of the urine. Only after complete suppression of urine as a result of massive doses of acid do granules of the sort first mentioned manifest the color indicative of acidity.

THE AUTOPLASTIC TRANSPLANTATION OF TISSUES INTO THE BONE MARROW CAVITY. MAURICE N. RICHTER and HENRY L. JAFFE, J. Exper. Med. **47**: 981, 1928.

The thymus when transplanted autoplastically into the bone marrow undergoes complete but not abundant regeneration. The histologic structure of the regenerated thymus is the same as that of the normal thymus despite its situation in the marrow cavity. Metaplasia of the thymic round cells into myelocytes was not noted. The regeneration of the bone marrow in the curetted cavity is not influenced by the presence of the thymus. The regeneration of the thymus is accompanied by its enclosure in a fibrous and bony capsule which prevents extensive development and causes pressure atrophy of the transplant.

AUTHORS' SUMMARY.

THE PATHOGENESIS OF EARLY OBSTRUCTIVE JAUNDICE. E. S. GUZMAN BARRON and JOHN H. BUMSTEAD, J. Exper. Med. **47**:999, 1928.

After experimental ligation of the bile ducts in dogs, two distinct processes are clearly manifested: (1) the accumulation of the normally circulating bilirubin in the blood with its characteristic indirect van den Bergh reaction for a period of several hours, and (2) the subsequent appearance of the bile bilirubin giving the

direct van den Bergh reaction. It is possible that the first process may be due to a temporary reflex inhibition of the function of the liver cells owing to ligation of the duct and comparable to the same phenomenon which usually occurs in the kidney when the ureter is ligated. The second process begins before any rupture of the bile capillaries is visible. Sections of the liver made from six to seven hours after obstruction show these bile capillaries dilated and extending between the liver cells in small distended pouches, the blind end of these lying in contact with the pericapillary spaces. It is possible that bile may diffuse from these thin walled pouches into the perivascular lymph spaces, this diffusion being favored by the mounting pressure inside the bile ducts. In early obstructive jaundice bile first appears in the lymph, but exclusion of the thoracic duct from the circulation by drainage causes a delay of only a few hours in the appearance of bile bilirubin in the blood stream. It must, therefore, be concluded that after obstruction of the biliary tract, bile enters the circulation by way of both the blood capillaries and the lymphatics, although the latter route is the most important.

AUTHORS' SUMMARY.

STENOSIS (COARCTATION) OF THE AORTIC ISTHMUS, WITH SUDDEN DEATH FROM RUPTURE OF A CEREBRAL ANEURYSM. F. PARKES WEBER, Proc. Roy. Soc. Med. **20**:1227, 1927.

This is a valuable contribution to this topic; excellent clinical and necropsy records with illustrations of the disease are found and indispensable references are given to the literature on this rare lesion. These all came about from discovering aortic coarctation with an opening barely 2 mm. in diameter as the cause of an aneurysm of a branch of the left cerebral artery. The aneurysm, 2.5 cm. in diameter, lay in the sylvian fissure. Aneurysms resulting from pressure proximal to the obstruction at the isthmus of the aorta are sometimes in the aorta where they may rupture and cause death. One other aneurysm of a cerebral artery due to aortic coarctation was reported by Woltman and Sheldon (*Arch. Neurol. & Psychiat.* **17**:302, 1927).

Weber suspected coarctation, because there were two pulsating superficial vessels, symmetrical, one on each side of the upper part of the front of the thorax in front, branches extending out toward the nipples from the greatly dilated internal mammary arteries. In two other patients he had previously correctly diagnosed coarctation from pulsating superficial arteries of the back of the trunk. He emphasized the clinical importance of absence of pulsation in arteries of the lower extremities. Intermittent claudication from interference with the cerebral circulation has been reported.

E. R. LE COUNT.

A STUDY OF OVARIAN METASTASES FROM CARCINOMAS OF THE DIGESTIVE TRACT. PAULETTE GAUTHIER-VILLARS, Ann. d'ant. path. **5**:1, 1928.

Gauthier-Villars reports ten cases of the so-called Krukenberg tumor of the ovary. The ovarian tumor is now established to be metastatic and not primary. The author quotes Rau who found that of eighty-six gastric cancers nine metastasized to the ovary, of eight cancers of the colon, one metastasized to the ovary, of thirty cancers of the biliary passages and thirty-six of the breasts, three and nine metastasized to the ovaries, respectively. The structure of the tumor is polymorphous, having a glandular, solid or mixed architecture. An essential feature of the epithelial cells is their formation of mucus. Besides the ovary, secondary nodules are also occasionally present in the uterus and tubes, and even in the bones. The metastases spread apparently by way of the lymphatics and also by transperitoneal dissemination. The author gives a detailed report of ten new cases and reviews the literature in a comprehensive way. The article is accompanied by an extensive bibliography.

B. M. FRIED.

CONCERNING SOME TYPES OF OSSEOUS SCLEROSIS. C. RUPPE, Ann. d'anat. path. **5**:41, 1928.

A critical review is given of the modern theories on fibrous changes found in the bones. The author takes up in great detail the pathologic changes of so-called pure sclerosis of the bones, the von Recklinghausen type of sclerosis, leontiasis ossea and, finally, Paget's disease. The discussion is comprehensive and is illustrated by case histories. The bibliography is extensive.

B. M. FRIED.

A CASE OF PERNICIOUS ANEMIA. A. M. COYON and C. BRUN, Sang **1**:437, 1927.

In a case of a cryptogenetic anemia in a girl, aged 25, the authors found pronounced erythropoietic activity in the removed spleen. Instead of its usual rôle in the destruction of red cells, this organ then assisted the bone marrow in its regenerating effort.

B. M. FRIED.

SIDEROUS SPLENOGRANULOMATOSIS. CARLA GAMA, Sang **1**:610, 1927.

The disease described by the French workers under the name of primary aspergillar splenomegaly was identified for the first time by Gama in 1922. Outside of Italy it was found in France, Algeria, Germany, Switzerland and South America. Nothing new has been added to the morphology of the lesion, and the specific nodule found in the enlarged spleen is designated by continental writers as Gama's nodule. Emile-Weil and his associates, Nanta and Pinoy, respectively, attribute the lesion to an aspergillus. Gama does not share the opinion of these writers. He states that what is identified as an aspergillus is merely "the result of a gradual regressive alteration of the connective tissue." Gama examined numerous spleens including sections from spleens removed by Emile-Weil and by others. He emphatically states that there is no such thing as an aspergillus which causes the splenomegaly. His article is provided with photomicrographs supporting his thesis. The pathogenesis of the disease, which is apparently a separate entity, is as yet obscure.

B. M. FRIED.

PULMONARY EMBOLISM; A STUDY OF THE ETIOLOGY OF ITS INCREASE. F. HÖRING, Deutsche Ztschr. f. Chir. **207**:360, 1928.

The author considers embolism of the lung the primary concern of the pathologist, although most of the contributions in the literature are made by surgeons and obstetricians. The criteria he employs to determine death by embolism are: (1) the embolus must obstruct one third of the circulation of the lung and (2) death must occur with a sudden insufficiency in the respiratory or circulatory functions. Cognizance is taken of the fact that small emboli may bring about death when the heart is already weak; also that emboli may act as a cause of death secondarily in those instances in which infarction already exists as a result of cardiac weakness, pulmonary stasis or small septic emboli. From Jan. 1, 1922, to Sept. 12, 1927, 3,248 autopsies revealed death from pulmonary embolism 131 times. After doubtful instances were deducted, the percentage in 1922 was 1.5 and in 1927, 5.6; there were 63 males and 68 females. Divided into broad groups 51 deaths occurred after operations, 5 followed childbirth, 10 followed fractures and other traumas and 65 occurred in medical patients without surgical intervention. Most deaths occurred between the third and fourth days after operation in the first group, in contrast to the fifth and sixth days as recorded in most statistics. Resections of the stomach, gastro-enterostomies, gynecologic procedures, prostatectomies and herniotomies preceded the vast majority of deaths in this group. Etiologically, infection played a major rôle in determining the formation of thrombi; cardiac and arterial changes ranked next and then obesity. In the medical group, cachexia was also pronounced for fifteen of the sixty-five

patients had cancer. Probably additional causative factors advanced are: alterations in the colloidal chemistry of the blood and injury to the patients by clinical treatment.

GEORGE RUKSTINAT.

THE DRAINAGE PATHS OF THE SUBMENINGEAL SPACES OF THE SPINAL CORD. GEORG IWANOW, Ztschr. f. d. ges. exper. Med. **58**:1, 1927.

The injection of filtered dilute india ink into the subarachnoid space of dogs under pressure of from 30 to 50 cm. of water, led (within twenty-four hours or less) to the appearance of ink in near (retroperitoneal, aortic), as well as distant (mesenteric, mediastinal, deep cervical) lymph nodes and lymphoid masses (Peyer's patches). The bulk of the ink was retained within the arachnoid by phagocytic cells, and, mechanically, by the partitions of the subarachnoid spaces. The experiments suggest anatomic connections between the subarachnoid space and various lymph nodes. If the subarachnoid space was divided by a ligature or by tying a strip of muscle around the cord (in its cervical or thoracic portion), the injection of lymph nodes was more complete; this was interpreted as due to compensatory dilatation of lymph channels. Iwanow concludes that the cerebrospinal fluid is drained from the subarachnoid space by lymph channels; foreign substances introduced into the subarachnoid spaces are partly retained by the arachnoid cells and partly removed by the lymphatics; they may also be removed by the diploic veins through the pacchionian bodies.

B. LUCKÉ.

AN ATTEMPT TO EXPLAIN THE MECHANISM OF SPONTANEOUS HEMORRHAGES IN SCURVY. W. M. NOWODWORSKI, Ztschr. f. d. ges. exper. Med. **58**:424, 1927.

In fourteen cases of scurvy, the number of blood platelets was somewhat decreased (average 60 per cent of the normal value); there were no changes in clotting time; calcium and magnesium contents were somewhat decreased. The values for fibrinogen and for thrombin were within normal limits. Contractility of the clots was normal. The author believes that the cause of spontaneous hemorrhages in scurvy lies in changes of the vessel walls.

B. LUCKÉ.

EXPERIMENTS ON VITAL STAINING OF LUNGS OF SURVIVING DOGS. G. SEEMANN, Ztschr. f. d. ges. exper. Med. **58**:434, 1927.

Heart-lung preparations (after the method of Starling) were perfused (*a*) with defibrinated blood and (*b*) with oxygenated Ringer-Locke solution; to these perfusing fluids filtered lithium carmine was added. Control animals received an intravenous injection of the same solution of dye. The duration of the experiments was from about one to two hours. Smears were made from the cut surfaces of the various organs; in addition, paraffin and frozen sections were studied. In the control animals carmine granules were found in the histiocytes of various organs and in polymorphonuclear leukocytes. The appearance of the dye granules was the same in the two classes of phagocytic cells; they were partly small rose-red granules and partly larger clumps. Such rose-red stained clumps were also abundantly free in the capillaries of the liver, lung, spleen and other organs. These stained clumps were regarded as coagulated plasma, and not as carmine precipitates. In Starling's heart-lung preparation (perfused with blood) similar pictures were obtained. After perfusion with Locke-Ringer solution few carmine-containing phagocytes were encountered; there was, however, a diffuse staining of the tissues. The writer regards vital staining of histiocytes and leukocytes with carmine as a phagocytosis of carmine-stained serum (or plasma) coagulates, and not as direct ingestion of carmine granules. The occurrence of phagocytic histiocytes in the alveolar septums in the Starling heart-lung preparation is regarded as proof for their normal occurrence in this location.

B. LUCKÉ.

CHANGES IN THE GREAT VESSELS OF SYPHILITIC RABBITS. S. BERGEL, Ztschr. f. d. ges. exper. Med. **58**:515, 1927.

Descriptions and photographs are presented of aortic lesions of syphilitic rabbits which resemble the changes characteristic of syphilitic aortitis in man. In addition, various other vascular lesions are described (calcification of media of aorta, etc.), the etiology of which is undetermined.

B. LUCKÉ.

THE ANATOMIC CONNECTIONS OF THE CEREBRAL AND SPINAL SUBMENINGEAL SPACES WITH THE LYMPATHIC SYSTEM. G. IWANOW and R. ROMODANOWSKI, Ztschr. f. d. ges. exper. Med. **58**:596, 1927.

From experiments on living and dead dogs it was concluded that the cerebrospinal fluid is drained by three separate pathways: the veins, lymphatics which come off directly from the meninges and through communication of the sheaths of certain nerves with lymphatics.

In the course of drainage various lymph nodes are traversed, and the fluid is mixed with lymph. Eventually the fluid is collected in the large lymphatic ducts (thoracic duct, etc.).

B. LUCKÉ.

ANEMIA IN SPRUE. A. KRJUKOFF, Folia haemat. **35**:329, 1928.

From a large number of cases of sprue, Krjukoff reached conclusions as follows: The blood in this disease is usually hyperchromatic or normochromatic. Under the influence of treatment it may become hypochromatic. The changes in the red blood cells are degenerative. The bone marrow in the ribs shows a megaloblastic reaction with the formation of megaloblasts and normoblasts. There is a leukopenia and a lymphocytosis; the number of monocytes and eosinophils is increased. The anemia in sprue is of the hemolytic type and is closely related to pernicious anemia.

B. M. FRIED.

THE RETICULO-ENDOTHELIAL SYSTEM (INCLUDING HODGKIN'S DISEASE, LYMPHOSARCOMA AND THE ALEUKEMIAS). REVIEW OF THE AMERICAN LITERATURE FOR 1926. CECIL J. WATSON, Folia haemat. **35**:369, 1928.

LEPROUS CHANGES IN TESTIS, EPIDIDYMIS AND SPERMATIC CORD. W. KOBAYASHI, Acta Dermat. A. Series, no. 2, Imperial University of Kyoto, Japan, 1928.

The results of the examination of some eighteen cases of leprosy are reported. The testis, epididymis and spinal cord were practically always affected bilaterally with more or less typical changes of the inflammatory granulating tissue type. *Bacillus leprae* was present in large numbers, especially in the testis and epididymis.

## Pathologic Chemistry

CALCIUM AND INORGANIC PHOSPHORUS IN THE BLOOD OF RABBITS. WADE H. BROWN, J. Exper. Med. **47**:539 and 563, 1928.

Determinations of calcium and inorganic phosphorus were made on the blood of four groups of normal rabbits at intervals of from one to two weeks over periods of from four to eight months. From the data thus obtained, values were calculated for calcium and inorganic phosphorus, and for various relations between the two substances. Determinations of calcium and inorganic phosphorus were made on the blood of a large series of rabbits as they were received from the dealer for the purpose of determining whether results obtained under these conditions differ from those obtained by repeated observations on animals living in the laboratory. It was found that the results differed in the two cases, owing largely to a decided difference in the inorganic phosphorus content of the blood; but the values for calcium were also affected.

AUTHOR'S SUMMARY.

CALCIUM AND INORGANIC PHOSPHORUS IN THE BLOOD OF RABBITS. WADE H. BROWN, J. Exper. Med. **47**:637, 1928.

Determinations of calcium and inorganic phosphorus were made on the blood of five groups of normal animals over periods of from four to eight months. The material included animals from recently acquired stocks as well as animals that had been living under laboratory conditions for long periods of time. The results were analyzed with especial reference to the occurrence of periodic and progressive variations in the absolute amounts of calcium and inorganic phosphorus and the relations between the two substances. It was found that, among animals living in the laboratory, both calcium and inorganic phosphorus, as well as all expressions of the equilibrium between the two substances, exhibited a definite tendency to a progressive increase or decrease, as the case might be, and that clearly defined periodic variations occurred in all classes of animals. The progressive change was most marked in the case of inorganic phosphorus, the periodic change in the case of calcium, while both conditions were clearly shown by various expressions of the relation between calcium and inorganic phosphorus.

AUTHOR'S SUMMARY.

CHANGES IN THE HYDROGEN ION CONCENTRATION DURING SLEEP. JOHANN KUNZE, Ztschr. f. d. ges. exper. Med. **59**:248, 1928.

The hydrogen ion concentration of the blood during sleep increases about 10 per cent (this corresponds to a lowering of the $p_H$ to between 0.02 and 0.06).

B. LUCKÉ.

THE ISO-ELECTRIC POINTS OF THE BLOOD WITH SPECIAL REFERENCE TO SYPHILIS. B. LUCHSINGER, Ztschr. f. d. ges. exper. Med. **59**:393, 1928.

A technic for determining the iso-electric points of complex protein systems, such as body fluids, has been elaborated by Vlès and de Coulon (for the lengthy references to their articles, the original paper should be consulted). According to these authors it is probable that in body fluids the several proteins do not exist as independent chemical entities but form complexes. The iso-electric point of these complexes lies in the vicinity of the mean of the iso-electric region of the several proteins that form the complexes. In normal serum four iso-electric points have been determined; the most stable lies at $p_H$ 5.5 and is designated as iso-electric point $a$. The authors cited have shown a close relationship between the iso-electric point of the serum, and various flocculation reactions and the sedimentation rate of erythrocytes. It has also been found that the iso-electric points of the serum shift in different pathologic conditions, such as acute infectious diseases. In cases of active syphilis Luchsinger found a quantitative parallelism between the flocculation reaction of Vernes, the sedimentation rate of erythrocytes and the position of the iso-electric point $a$. This parallelism occurred throughout the course of the disease; under specific treatment all three phenomena tended to approach the normal. However, no such relationship was found in case of chronic or latent syphilis, or in syphilis of the nervous system. In such cases the iso-electric point and the sedimentation rates of erythrocytes were but little altered, while the reaction of Vernes gave increased values. It is interesting that in these cases specific treatment did not change the reactions to any considerable extent. The author interprets the shifting of the iso-electric points of the serum in different diseases as the result of a nonspecific general reaction of the tissues (proteins). It is possible that such general reactions occur only during the more acute course of infections.

B. LUCKÉ.

EFFECT OF $p_H$ OF BLOOD ON NUMBER OF ERYTHROCYTES. S. WEISS, F. KISS and N. KUSZING, Ztschr. f. d. ges. exper. Med. **60**:58, 1928.

Six cubic centimeters of an 8 per cent solution of sodium bicarbonate was given daily through a stomach tube to guinea-pigs (ten) for four weeks. The $p_H$

of the blood changed from 7.58 to 7.76. Another series (ten) received 6 cc. of fifth normal hydrochloric acid for the same period; the $p_H$ of the blood changed from 7.58 to 7.45. The number of erythrocytes and leukocytes and the percentage of hemoglobin were determined in the usual manner. There was a definite increase in the number of erythrocytes (from about 500,000 to 1,000,000) in the animals to which hydrochloric acid had been administered; in the animals that had been alkalinized a decrease of erythrocytes (roughly 500,000) occurred. The percentage of hemoglobin decreased in both groups. The number of leukocytes increased in the animals to which hydrochloric acid had been given (on an average about 4,000); in the other group definite changes did not occur. The authors suggest that the changes in the number of erythrocytes which occur in a number of diseases are possibly related to slight shift of the $p_H$ of the blood.

B. LUCKÉ.

THE PROTEINS OF THE BLOOD IN VARIOUS DISEASES. WILHELM STARLINGER and ELSBETH WINANDS, Ztschr. f. d. ges. exper. Med. **60**:138, 160, 185 and 208, 1928.

In a series of four papers are given the results of the determination of plasma volume and of the albumin, globulin and fibrinogen content of the blood of 244 healthy and diseased subjects. The results obtained are compared with those reported in the literature. The following mean normal values are given (in round figures): total proteins, 8 Gm. per hundred cubic centimeters; fibrinogen, 0.25 Gm. per hundred cubic centimeters = 4 per cent of total proteins; globulin, 2.5 Gm. per hundred cubic centimeters = 35 per cent of total proteins; albumin, 5 Gm. per cubic centimeters = 62 per cent of total proteins; plasma volume from 51 to 61; average, 58 per cent. The range of normal values (25 determinations on 16 persons between the ages of 20 and 32; most of them females) were: total plasma proteins from 6.93 to 9.13 Gm. per hundred cubic centimeters; fibrinogen from 0.22 to 0.36; globulin from 1.41 to 4.03; albumin from 4.21 to 5.78 Gm. per hundred cubic centimeters. Values are given for the following diseases: diphtheria, scarlet fever, measles, typhoid, influenza, sepsis, pneumonia, fibrinous bronchitis, bronchiectasis, tuberculosis, syphilis, malaria, rheumatic fever, cardiac diseases, renal diseases, diseases of the hemolyticopoietic organs, diseases of the liver, malignant tumors, leukemia, Hodgkin's disease, gastric ulcer, gastro-enteritis, diabetes and other diseases of the organs of internal secretion, and diseases associated with serous and with purulent effusion. Spontaneous fluctuations in conditions of health and disease are also discussed. While it is not possible to abstract the extensive data given, the papers will serve as a valuable reference.

B. LUCKÉ.

## Microbiology and Parasitology

CONCERNING THE TUBERCULOUS NATURE OF PLEURISY FROM THE LIFE INSURANCE STANDPOINT. H. B. ANDERSON, Am. Rev. Tuberc. **17**:147, 1928.

Pleurisy occurs rarely as a primary disease. The frequent evidences of pleurisy found at autopsy, contrasted with the relative infrequency of clinical pleurisy, suggest that pleural adhesions are often the result of an inflammation that is clinically latent. Ordinary pleurisy with effusion is practically always tuberculous. Ordinary dry pleurisy is usually tuberculous, but this group includes a number of cases of pleurodynia, myositis, herpes zoster and intercostal neuralgia, and a number of cases secondary to focal infections, rheumatism, septicemia, emboli, bronchitis, bronchiectasis and pneumonokoniosis. Pleurisy represents a relatively mild and curable phase of tuberculosis. A completely recovered focal tuberculosis in childhood provides an increased immunity against tuberculosis in later years.

H. J. CORPER.

THE DIAGNOSIS OF TUBERCULOSIS BY MEANS OF THE HAMSTER. JOHN H. KORNS and GEORGE Y. C. LU, Am. Rev. Tuberc. **17**:279, 1928.

The striped hamster (*Cricetulus griseus*) a small field rodent weighing about 30 Gm. and normally inhabiting ground burrows was used for diagnosis of tuberculosis. This rodent is easily purchased in the market in Peking, being supplied by farmers. The lesions are smaller than in guinea-pigs and, therefore, less striking in the gross. The lungs usually show the most characteristic lesions, while the tracheobronchial lymph nodes and spleen are perceptibly, often markedly, enlarged. Microscopically, the lesions are almost wholly proliferative, there being less caseation than in the guinea-pig and only slight tendency toward giant cell formation. Tubercle bacilli are usually found in abundance in the lesions.

H. J. CORPER.

THE CULTIVATION OF TUBERCLE BACILLI WITHIN DIFFUSION MEMBRANES IN VITRO AND IN VIVO. P. C. HOU, Am. Rev. Tuberc. **17**:422, 1928.

Excellent growth of tubercle bacilli takes place within diffusion membranes, including cellulose-casing (purified cellulose) celloidin and parchment, without the passage of bacteria into the surrounding medium. In six weeks' time tuberculo-protein, as shown by biologic and chemical tests, passes into the medium surrounding the membrane. Good growth occurs on ground human tissue when diffusion membrane separates the tissue and the growing tubercle bacilli. In a few instances, the cultivation of tubercle bacilli has been achieved on a small scale inside of diffusion membranes embedded subcutaneously and intraperitoneally in guinea-pigs.

H. J. CORPER.

DIFFERENTIAL JUGULAR BLOOD CULTURES IN SINUS THROMBOSIS. REUBEN OTTENBERG, J. A. M. A. **90**:1602, 1928.

Differential jugular blood cultures consist of simultaneous cultures from both internal jugular veins (and preferably also from an arm vein). The procedure is simple and safe, as indicated by the results in fifteen cases. In cases of thrombosis of the lateral sinus, the blood from one jugular vein usually shows a far larger number of colonies than that from the other jugular vein or from the arm vein. In the more common instance in which the thrombus completely occludes the sinus, the larger number of bacteria are found not in the blood from the diseased side but in that from the normal side. In the less frequent instance of a mural, nonocclusive thrombus, the larger number of bacteria are found on the diseased side. The cases cited show that the bacteria are killed in or removed from the circulation blood with enormous rapidity.

AUTHOR'S SUMMARY.

TWO CASES OF DIPHYLLOBOTHRIUM LATUM INFESTATION IN MASSACHUSETTS. EDWARD G. MCGAVRAN and MAHIDOL SONGKLA, J. A. M. A. **90**:1607, 1928.

The article concerns two children of Russian Jewish parentage who occasionally ate raw fish. The chief supply of the fish concerned came from the region of the Great Lakes and the Ohio river.

BRUCELLA ABORTUS INFECTION IN MAN. R. L. SENSENICH and A. S. GIORDANO, J. A. M. A. **90**:1782, 1928.

The characteristic symptoms of *Brucella abortus* infection in man are anorexia; loss of weight and strength; headache, and chills, fever and sweats of varying intensity throughout long periods of time, frequently with intermissions. The fever has a tendency to exhibit undulations which may vary in character and in length. Evidence of the effect of the disease on the nervous system is constant, and arthritis is a common symptom.

The consideration of *Brucella abortus* infection in the differential diagnosis of all conditions exhibiting variable combinations of these symptoms will undoubtedly reveal more frequent occurrence of this disease.

Isolation of the organism from the blood or a positive agglutination reaction makes the diagnosis definite, although the disease may be present with negative manifestations.

A Segregation Method for Eliminating Tuberculosis from Cattle. C. R. W. Adeane and J. F. Gaskell, J. Hyg. **27**:248, 1928.

The tuberculin reaction properly applied is a reliable test for the presence of tuberculosis in cattle. A method of segregation is described by which any herd can be rendered tuberculin negative in from three to four years without dimunition of the herd. Evidence is given that recovery from a tuberculous infection can take place in cattle. Calves can be infected with tuberculosis only after birth; such an infection may, however, take place extremely rapidly within the first few days of life. Artificial feeding of calves with sterilized milk must be supplemented by some vitamin-containing food; otherwise "joint ill," corresponding to rickets in human beings, will develop at about the third or fourth month. Bull calves are more susceptible to joint ill than heifers.

Authors' Summary.

Experimental Inoculation of Chickens with Hodgkin's Nodes. Elsie S. L'Esperance, J. Immunol. **15**:123, 1928.

This is a preliminary report on five chickens that developed tuberculosis after intravenous injections of emulsions of lymph nodes from two cases of Hodgkin's disease. Further work is in progress.

The Twort-d'Herelle Phenomenon. A Critical Review and Presentation of a New Conception (Homogamic Theory) of Bacteriophage Action. Philip Hadley, J. Infect. Dis. **42**:263, 1928.

The recent experiments and observations dealing with the Twort-d'Herelle phenomenon are reviewed and, in preference to the theories of Kabeshima, Bordet and Ciuca, and d'Herelle, a working hypothesis is evolved — that the bacteriophage is either a definite stage in the cyclogeny of the bacterial species, or a functionally active particle accessory to one of these stages. The term accessory means possessing complementary or reciprocal biologic significance as exemplified, for instance, in the relation of sperm cell to ovum. In such a conception there is no priority of significance in the relationship between bacteriophagic corpuscle and the cell that generates it, or between the corpuscle and the cell which it attacks. Both elements are necessary components of a definite reproductive mechanism possessed by many, if not by all, bacteria. This different interpretation is determined by the author's point of view on the question of monomorphism and the reproductive behavior of bacteria. According to this conception the future views, not of transmissible autolysis alone, but of several other highly important aspects of bacterial behavior must be rebuilt on a newer and firmer foundation — the clear recognition of the far-reaching significance of bacterial cyclogeny and the dissociative reactions which are natural and inevitable consequents.

Author's Summary.

The Placental Transmission of Bacteriophage. John E. Blair and David L. Reeves, J. Infect. Dis. **42**:440, 1928.

It has been possible to demonstrate the placental transmission in guinea-pigs of three strains of bacteriophage of high potency, following intraperitoneal or subcutaneous inoculation. Attempts to demonstrate placental transmission of bacteriophage using strains of low potency were negative. Experiments were negative in which the bacteriophage was administered by mouth, although it was shown that feces of the mother contained the bacteriophage immediately after feeding.

Authors' Summary.

MOTILITY OF CLOSTRIDIUM BIFERMENTANS. W. S. STURGES and E. T. DRAKE, J. Infect. Dis. **42**:446, 1928.

In young cultures in veal extract broth both *Clostridium bifermentans* and *C. centrosporogenes* were found to be actively motile when examined by a special flattened capillary method which allows little exposure to air. A close relationship between these organisms is indicated.

AUTHORS' SUMMARY.

THE ACTION OF BACTERIOPHAGE IN PRODUCING FILTRABLE FORMS AND MUTATIONS OF BACTERIA. MARTIN FROBISHER, J. Infect. Dis. **42**:461, 1928.

In the experiments described in this paper no evidence was found of the occurrence of ultrabacteria due to the action of bacteriophage. The secondary growths observed are regarded as the result of defective filters, poor stoppers, insufficient autoclaving or technical error.

Filtration with filters of the Berkefeld type is an operation in which a variety of obscure sources of error may vitiate the results. Filters may appear perfect when tested with air pressure yet allow the passage of ordinary bacteria. This passage may at first occur only occasionally and pass unnoticed unless close check is kept on the identity of the filter and its products.

The conclusions of Bronfenbrenner and Muckenfuss are confirmed.

AUTHOR'S SUMMARY.

AN INVESTIGATION OF THE RELIABILITY OF SPUTUM TYPING OF PNEUMOCOCCUS BY THE MOUSE METHOD. W. D. SUTLIFF, J. Infect. Dis. **42**:485, 1928.

The mouse method of sputum typing has been found reliable in 97 per cent of the cases in which a fixed type of pneumococcus (1, 2 or 3) was reported.

The mouse method of sputum typing has been found reliable in 85.2 per cent of the cases in which a pneumococcus type 4 was reported.

The precentage of error in pneumococcus type 4 group can be largely eliminated when cultures are taken from other parts of the patient (spinal fluid, empyema fluid, joint fluid, pericardial fluid, abscesses, cultures made at necropsy, including lung cultures, blood cultures) or when a second specimen of sputum is examined by the mouse method.

AUTHOR'S SUMMARY.

EFFECT OF REDUCED OXYGEN TENSIONS ON THE GROWTH OF CLOSTRIDIUM BOTULINUM (TYPE A) IN VEAL INFUSION BROTH. GAIL M. DACK and M. MEREDITH BAUMGARTNER, J. Infect. Dis. **42**:491, 1928.

*Clostridium botulinum*, type A, was grown in veal infusion broth under reduced oxygen pressures. No growth was obtained when the oxygen pressure was greater than 1.3 cm. As the oxygen pressures increased from 0.1 to 1.3 cm., the time of growth became more irregular.

AUTHORS' SUMMARY.

GAS PRODUCTION FROM COMMERCIAL PEPTONES BY B. AEROGENES AND B. COLI. E. LEE TREECE, J. Infect. Dis. **42**:495, 1928.

Gas was produced from two of the four commercial peptones tested by *B. aerogenes* and *B. coli*, both in a liquid medium under petrolatum and in a peptone medium solidified with agar or gelatin.

The gases produced by *B. coli* and *B. aerogenes* were collected, measured and analyzed. The gas ratio for *B. aerogenes* was found to average 8.5, varying from 5.7 to 12.8. The gas ratio for *B. coli* was infinitely higher: the value could not be determined since the amount of hydrogen produced was too small to be measured or analyzed. This wide variation indicates fundamental differences in the modes by which *B. coli* and *B. aerogenes* break down the gas-producing substance of commercial peptone.

Evidence presented in this paper indicates that the source of this gas is not in the carbohydrate radicles of the peptone.

AUTHOR'S SUMMARY.

SANITARY SIGNIFICANCE OF LACTOSE-FERMENTING BACTERIA NOT BELONGING TO THE B. COLI GROUP. FRANK E. GREER, J. Infect. Dis. **42**:501, 1928.

A review of the literature discloses reports of several types of lactose-fermenting bacteria isolated from water or from sources indicating their presence in water. The most common are *Clostridium welchii, Bacillus aerosporus* (n. sp. Norton) and *Streptococcus fecalis.* There are also types less frequently found in isolated localities. These include the leather bacillus of Houston, *Phytomonas aerogenes* (n. sp. Butler), and an anaerobe described by Raab.

We have isolated and identified several of these organisms in the Chicago water supply, including *C. welchii, B. aerosporus, Streptococcus fecalis,* the "leather bacillus," and members of the mucosus-capsulatus group.

The most common lactose-fermenting organisms found in Lake Michigan water are *C. welchii, B. coli, B. aerosporus, Streptococcus fecalis* and two undetermined groups of organisms, designated as Bo and To.

The numbers of these organisms show a seasonal variation and also a variation as between raw and treated water. They often render the forty-eight hour presumptive test of treated water valueless as an index of *B. coli* during many months of the year.

AUTHOR'S SUMMARY.

PROVOCATIVE ACTION OF ORGANIC COMPOUNDS OF ANTIMONY IN LEISHMANIASIS. R. N. CHOPRA and C. R. DAS GUPTA, Indian J. M. Research **15**:565, 1928.

Animal experiments show that injections of antimony compounds produce a well marked increase in the volume of such organs as the liver and the spleen, and that there is also a remarkable increase in the rhythmic movements of these organs. That a similar state of affairs is brought about in man, after intravenous injections of these compounds, is shown by the fact that most of the patients complain of a feeling of fullness and discomfort after the injection. This is further borne out by the fact that in many cases the engorgement and increased rythmic movements produce rupture of the endothelial cells and liberation of Leishman-Donovan bodies into the general circulation. The organisms therefore appear in the blood of these patients in whom they could not be detected before. Injections of antimony compounds, therefore, have a provocative action in those cases of kala-azar in which the fibrotic changes in the liver and the spleen are not too advanced.

AUTHORS' SUMMARY.

RAT LEPROSY. E. MUIR and J. M. HENDERSON, Indian J. M. Research **15**:807, 1928.

Rats can be infected with rat leprosy by the scarification, subcutaneous and intraperitoneal routes of inoculation. In connection with the two last mentioned routes, spread of the infection has been demonstrated as early as about the third week following inoculation, while microscopically evidence of cellular proliferation at the site of a subcutaneous inoculation has been detected as early as the eighth day thereafter. The infrequency of lesions within the kidneys and the suprarenals has been commented on and the occasional production of granulomatous tumor-like masses after the intraperitoneal route of inoculation has been noted. Increased virulence of infection does not occur in rats fed on vitamin-deficient diets, or on a diet rich in protein-decomposition products. Lesions can be produced in a small number of cases after feeding rats with infected tissue; but the incubation period is a long one, and the results are capricious. No lesions comparable to those seen in the rat have been obtained in the monkey, the rabbit or the Chinese hamster after the inoculation of rat leprosy material. Rat leprosy bacilli are rendered nonvirulent by exposure to a temperature of 60 C. for twenty-five minutes or longer. Inoculation of human leprosy material into rats, Chinese hamsters and Japanese dancing mice gave completely negative results. Calmette's B. C. G. culture does not have any effect in protecting rats against subsequent infection with rat leprosy bacilli.

AUTHORS' SUMMARY.

THE SPIROCHAETA NORMANDI. CHARLES NICOLLE, CHARLES ANDERSON and JACQUES COLAS-BELCOUR, Arch. de l'Inst. Pasteur de Tunis **17**:1, 1928.

This recently discovered spirochete was isolated from a Tunisian tick, *Orn. normandi* (photographs and entomologic description in an article immediately succeeding, p. 35). Mice are easily infected, maintaining the organisms in their blood for a period up to thirty-six days. The malurus, a bird (*Meriones shawi*), acts as a reservoir for the virus. White rats and rabbits are more or less susceptible; guinea-pigs, dogs, goats, pigeons, and lizards, not at all. Differentiation from other species of spirochetes on this basis is possible. In monkeys a recurrent fever is produced, with numerous spirochetes present. Man is susceptible, but has an "inapparent infection" (in the single case recorded). Infected mice recover with an immunity lasting from one to two months. There is no consequent immunity to infection with other species. A similar spirochete was isolated in Carthage, which the authors consider a separate strain of the same species.

M. S. MARSHALL.

CYTOLOGIC STUDIES OF PALUDISM. C. W. ANDERSON and E. V. COWDRY, Arch. de l'Inst. Pasteur de Tunis **17**:46, 1928.

A study of the flagellation of *Plasmodium kochi* by means of the dark field is presented, illustrated by four color plates (119 figures). Monkeys were used as hosts to the parasite. The various stages in the formation of microgametes described are: (1) a latent period, of from one half to six minutes; (2) a stage of "gel" in which the Brownian movement of granular inclusions through the cytoplasm lessens from the periphery (ten seconds); (3) a penetration stage (thirty seconds) in which the parasite contracts, and protuberances appear and disappear; (4) an expansion stage (five seconds to ten minues) in which the microgametes loosen rapidly from one point or on all sides; (5) an expulsion stage (two seconds to five minutes) in which the microgametes become detached; (6) a curious transformation of free microgametes between the forward repant motion and the backward rotary motion, having nothing to do with obstacles in the path.

M. S. MARSHALL.

THE ACTION OF VITAMINS AND BIURET FREE EXTRACTS ON THE DEVELOPMENT OF B. DIPHTHERIAE AND B. INFLUENZAE. ADA DOMINICI, Boll. d. Ist. Sieroterapico Milanese **7**:137, 1928.

The favorable influence of several vegetable and animal substances rich in vitamin B (peas, yeast, rice hulls, pig's heart, bran, etc.) on the growth of *B. diphtheriae* and *B. influenzae* is greatly increased when such extracts are previously digested with pancreatine until biuret free. This is due in part to vitamin D which is associated with vitamin B, and, also, to the added amino-acids. The substances are not destroyed when autoclaved at a temperature of 130 C. for one hour. The stimulating action is markedly reduced when a temperature of 159 C. for one hour is applied.

A. J. SALLE.

THE STREPTOCOCCUS ANTIVIRUS OF BESREDKA. LOUROS and GAESSLER, Centralbl. f. Bakteriol. **104**:356, 1927.

The authors conclude that the growth-inhibiting properties of a streptococcus filtrate prepared according to the method of Besredka depend solely on the acid effect and are entirely nonspecific. The immunizing effect is merely that of nonspecific proteins. Consequently, this cannot be interpreted as an antivirus in the sense that Besredka considers it.

PAUL R. CANNON.

THE EFFECTS OF RABIES INFECTION ON RATS AND MICE. J. BROSCH, Centralbl. f. Bakteriol. **105**:192, 1928.

Brosch finds that rats and mice are susceptible to rabies injections, the incubation period averaging about fifteen days. Double injection (intracerebral and

intramuscular) gives the highest percentage of positive results. Symptoms of paralytic rabies develop in most cases. The Negri bodies are to be found in Ammon's horn. The author believes that mice and rats can be used to advantage in the diagnosis of suspected rabies material.

PAUL R. CANNON.

THE SIGNIFICANCE OF THE RETICULO-ENDOTHELIUM FOR THE GENERALIZATION OF VACCINE VIRUS. A. GOLDMAN, Centralbl. f. Bakteriol. **105**:333, 1928.

The injection of india ink in rabbits and guinea-pigs which had been injected subcutaneously with vaccine virus, caused a distinct prolongation of the time during which the virus could be demonstrated in the blood. The incubation period of the local reaction was also shortened. These results emphasize the importance of the reticulo-endothelium in the course of vaccine infection.

PAUL R. CANNON.

THE PRESENCE OF FRAENKEL'S GAS BACILLUS IN THE VAGINA OF NORMAL WOMEN. RICHARD SCHUBERT, Centralbl. f. Bakteriol. **105**:339, 1928.

The gas bacillus was found in the vaginal secretion of sixteen of twenty-five normal women examined. The importance of these observations in relation to abortion and the puerperium is discussed.

PAUL R. CANNON.

REACTION OF VESSELS OF THE ISOLATED EAR OF RABBIT TO INJECTION OF STREPTOCOCCI AND THEIR TOXIN. A. P. ANOCHINA-IWANOWA, Ztschr. f. d. ges exper. Med. **59**:352, 1928.

The vessels of the isolated ear of rabbit was perfused with Ringer-Locke solution containing living streptococci, or scarlet fever streptococcus toxins. The number of drops per minute of the perfusate indicated an inflammatory reaction (temporary vasoconstruction with subsequent vasodilatation, stasis and edema). These effects were more pronounced after perfusion with living streptococci than with streptococcus toxin. After perfusion with streptococci or with streptococcus toxins the vasoconstrictor effects of epinephrine and of caffeine were diminished.

B. LUCKÉ.

PASSAGE OF PARTICULATE MATTER THROUGH THE CAPSULE OF THE SPLEEN. H. ANTHES, Ztschr. f. d. ges. exper. Med. **59**:369, 1928.

The lower pole of the spleen of living guinea-pigs was placed in a small glass vial containing various substances (fat, carmine, cocci, tubercle bacilli), the passage of which through the splenic capsule was to be investigated. After a varying length of time, sections from the spleen were examined. It was found that particulate matter passes through the splenic capsule and into the substance of the spleen by means of lymphatics. These experiments suggest that bacteria and particulate matter in general may gain access to the spleen through its capsule.

B. LUCKÉ.

## Immunology

THE ANTIGENIC COMPLEX OF STREPTOCOCCUS HEMOLYTICUS. REBECCA C. LANCEFIELD, J. Exper. Med. **47**:843 and 857, 1928.

The anaphylactic reactions of two nontype-specific fractions of hemolytic streptococcus extracts parallel the precipitin reactions. The nucleoprotein, P, is a true antigen, in that it stimulates antibody production in rabbits, as shown before, and produces anaphylactic shock in guinea-pigs actively as well as passively. The probable carbohydrate, C, on the other hand, does not induce antibody formation in rabbits, so far as known at present, but does produce typical anaphylactic shock in guinea-pigs passively sensitized with antibacterial serum provided the serum shows

a high titer of C precipitins. This is an instance of a hapten, probably carbohydrate in nature, causing anaphylactic shock in passively sensitized guinea-pigs.

Type-specific anaphylactic shock was produced with hydrochloric acid extracts of *Streptococcus hemolyticus* in guinea-pigs passively sensitized with antibacterial serums. With occasional serums and certain hydrochloric acid extracts, type-specific shock was not produced unless the serum was first absorbed with heterologous bacteria, or unless the guinea-pigs were desensitized with heterologous extracts before testing with the homologous extract. The observations indicated that this was due to the presence of the nontype-specific substance which has been provisionally designated as Y. Tryptic digestion destroyed the ability of hydrochloric acid extracts containing the type-specific M substance and the non-type-specific Y substance to produce anaphylactic shock in passively sensitized guinea-pigs. Active sensitization was not accomplished with the type-specific M. It seems probable, therefore, that this substance is a hapten, reacting with antibodies but not stimulating their production after separation from the bacterial cell.

AUTHORS' SUMMARY.

THE PROPERTIES OF THE BACTERICIDAL SUBSTANCE IN MILK. F. S. JONES, J. Exper. Med. **47**:877, 1928.

Certain of the properties of the bacterial agent in milk have been studied. The substance is present in the colostrum and milk of the first few days of lactation as well as later. Its concentration varies in the secretion from various quarters of the same cow. Its activity is diminished by heat and cannot be restored again by the addition of active milk. The principle is present in whey and readily passes through the coarsest Berkefeld filter, although a considerable portion is retained by N candles. The finest filter (W) completely retains it. It is absorbed by animal charcoal but not by kaolin, kieselguhr or bolus alba. It can be desiccated, and its presence has been demonstrated in one brand of dried milk.

AUTHOR'S SUMMARY.

IS THERE AN IMMUNOLOGIC RELATIONSHIP BETWEEN HORSE SERUM AND HORSE DANDER? GEORGE F. FORSTER, J. Exper. Med. **47**:903, 1928.

Evidence has been submitted of the existence of a common antigenic substance in horse dander and horse serum. This evidence has been derived from three lines of study: (*a*) Cross-precipitation tests involving (1) the titration of antiserums against horse serum with saline extract of horse dander and (2) titration of antiserums against horse dander with normal horse serum; (*b*) cross-anaphylactic tests by the uterine strip method of Dale, and (*c*) cross-anaphylactic tests in living guinea-pigs by the usual shock method. It seems likely from the work here described that the common antigen is present in small proportion in horse dander. Its concentration in horse serum is not indicated by the results obtained.

AUTHOR'S SUMMARY.

IMMUNOLOGIC RELATIONSHIPS AMONG THE PNEUMOCOCCI. JOHN Y. SUGG, EMIDIO L. GASPARI, WILLIAM L. FLEMING and JAMES M. NEILL, J. Exper. Med. **47**:917, 1928.

The paper reports a study of a virulent, S-producing strain in pneumococcus which is immunologically related to, but not identical with typical strains of type III pneumococcus. In a potent antitype III serum, the relationship of this strain to typical III strains appears to be about the same as the relationship of Avery's subgroup type II strains to typical type II. But a more pronounced distinction is evident in the antiserum produced by immunization with the strain related to type III. This antiserum contained antibodies specifically reactive with typical type III bacteria as well as antibodies reactive with the homologous strain, while antisubgroup type II immune serums are devoid of antibodies reactive with typical type II pneumococci. The results of absorption experiments were the same as

those usually obtained with immunologically related, but not identical bacteria. The failure of reciprocal absorption and the marked variations in the relative potencies of the antiserum from different animals might be presented as presumptive evidence that two different anti-S antibodies are contained in type III immune horse serum. The theoretical significance of virulent pneumococci which are related to but not identical with the fixed types, is discussed from the standpoint of their importance in the biologic classification of the pneumococcus group.

AUTHORS' SUMMARY.

IMMUNOLOGIC RELATIONSHIPS AMONG THE PNEUMOCOCCI. ANNIE LUVERNE HARRIS, JOHN Y. SUGG and JAMES M. NEILL, J. Exper. Med. **47**:933, 1928.

The paper reports an experimental comparison of the antibody responses of mice and of rabbits to immunization with typical type III pneumococci and a strain related to type III. The results, as a whole, show that the relative antigenic effectiveness of these two kinds of related pneumococci, is different in different animals. In rabbits, the strain related to but not identical with type III elicits the better response; in mice, the typical type III strain is more effective. These relationships furnish an example of the influence of the species of animal on the effectiveness of even closely related bacterial antigens.

AUTHORS' SUMMARY.

SURFACE TENSION OF SERUM OF THE SENSITIZED GUINEA-PIG. SUSAN GRIFFITH RAMSDELL, J. Exper. Med. **47**:987 and 993, 1928.

The change in surface tension behavior in the serum of sensitized guinea-pigs is, as du Noüy has concluded for immunized rabbit serum, not referable to an antibody content, since it is known that the capacity for transfer of sensitization remains in the serum indefinitely, while the increased time-drop phenomenon is a transitory manifestation. That this phenomenon cannot be invoked by a new antigen capable of calling out its specific antibody would seem to make this response one due to some basic stable alteration of a tissue active in the general process of sensitization. That this alteration is not one called out by such a simple toxic injury as a uranium nitrate nephritis is contributory evidence that the primary toxicity of the horse serum is not the specific factor involved.

The primary change in surface tension of serum incident to anaphylactic shock is probably due to a lowering of the surface tension of the serum by the addition of the antigen serum. But this may be followed by a further decrease or by an increase depending on the intensity and duration of certain secondary tissue changes.

AUTHOR'S SUMMARY.

THE SPERMATOCYTE REACTION (TESTICULAR TUBERCULIN REACTION) IN THE ALBINO RAT. JOHN E. BLAIR, J. Infect. Dis. **42**:435, 1928.

The specific allergic response to tuberculin in the guinea-pig testis, described by Long as the "spermatocyte reaction," has been confirmed.

In the tests of albino rats on a normal diet, infected by intraperitoneal or intratesticular inoculation of human tubercle bacilli, the injection of dilutions of old tuberculin produces no allergic response.

AUTHOR'S SUMMARY.

SEARCH FOR PROTECTIVE, BACTERIOPHAGIC AND ENZYMATIC AGENTS IN PNEUMONIC SPUTUMS. BERNICE ELAINE EDDY, J. Infect. Dis. **42**:449, 1928.

In the majority of cases there is protection, or a latent period in the time of death, of mice given injections with fatal doses of pneumococci mixed with filtrates of sputum from patients with lobar pneumonia after crisis, as compared with control mice receiving only broth with the dose of pneumococci.

Protection or a latent period in the time of death of mice does not occur with filtrates of sputum obtained before crisis, with sputum filtrates from fatal cases,

with sputum filtrates after crisis injected with a pneumococcus of different type from that infecting the patient, nor with the sputum filtrate boiled and injected with homologous pneumococci.

A bacteriophage which might account for crisis was not recovered from the sputum.

Proteolytic enzymes which would erode Loeffler's blood serum as Lord described were demonstrated in the sputum of two patients with pneumonia.

AUTHOR'S SUMMARY.

THE HYDROGEN-ION CONTENT OF VACCINE LYMPH AND ITS RELATION TO THE POTENCY AND KEEPING PROPERTIES OF LYMPH PREPARED UNDER TROPICAL CONDITIONS. J. CUNNINGHAM and K. S. MHASKAR, Indian J. M. Research **15**:819, 1928.

The chemical reaction of vaccine lymph prepared and stored under tropical conditions has been investigated. Glycerinated lymph was used for the tests. Dialysis and centrifugalization were used to obtain a clear fluid for test purposes. In thirty-five freshly prepared batches of lymph the $p_H$ varied between 7.2 and 8, the majority lying between 7.4 and 7.8. After exposure to room temperature (98.4 F.) for varying periods, the majority of lymphs became more acid. At the same time there was a great reduction in the numbers of bacteria. The same tendency to an increased acidity was seen after the lymphs were treated with chloroform vapor for purposes of purification. When the reaction of different samples of the same batch of lymph were artificially adjusted to $p_H$ 4, 6, 8 and 10 and then stored at 55 F. and room temperature (average 98.4 F.) for different periods, it was found that the sample in which the reaction was adjusted to $p_H$ 8 gave the best results. The investigation shows that there is a tendency in most lymphs in the tropics to become more acid on storage or exposure, and, as the formation of acid has a deleterious action on the vaccine virus, the authors recommend that the reaction of each batch of lymph should be carefully adjusted to a point which is definitely alkaline, say $p_H$ from 7.6 to 7.8, to guard against deterioration from this cause.

AUTHORS' SUMMARY.

IMPOSSIBILITY OF VACCINATING THE GOAT AGAINST M. MELITENSIS. E. BURNET, Arch. de l'Inst. Pasteur de Tunis. **17**:73, 1928.

The author was not successful in immunizing goats by the injection of large amounts of bacterial suspension, either heated or treated with gold chloride.

M. S. MARSHALL.

CONCERNING THE LOCUS OF THE ANAPHYLACTIC REACTION. T. EHMER and J. HAMMERSCHMIDT, Klin. Wchnschr. **7**:931, 1928.

As a result of a specific septicemic infection in mice, a total but selective blockade of the vascular endothelium of the closed blood channels is accomplished, and anaphylactic shock in these animals is completely suppressed. This means that the vascular endothelium comes into consideration as a consequential tissue for anaphylactic shock. Further, there is no inhibiting substance in the blood of such infected animals, and anaphylaxis with septicemias caused by organisms which do not block the endothelial cells is not suppressed. Finally, the primary serum toxicity of foreign serums does not affect the vascular endothelium.

AUTHORS' SUMMARY.

BACTERICIDES OF THE INTESTINAL FLUIDS. K. MEYER and W. LOWENBERG, Klin. Wchnschr. **7**:984, 1928.

Bactericides of the intestinal fluids are not inorganic substances, proteins or other colloids of considerable dimension or lipoids, and they have slight surface tension

activities. Apparently, they have a large molecular constitution or are highly dispersed colloids. Presumably the bactericidal substances are secreted by the intestinal mucosa.

EDWIN F. HIRSCH.

SENSITIZATION TOWARD DEFINITE CHEMICAL SUBSTANCES. W. FREI, Klin. Wchnschr. **7**:1026, 1928.

Minute doses of neoarsphenamine (from 0.001 to 0.0075 mg.) injected intracutaneously sensitized the entire skin of guinea-pigs toward neoarsphenamine. This sensitization of the skin in animals was limited at first to a small percentage, comparable to that in human beings. After several weeks, almost all the animals became sensitive, although there was some uncertainty that the early reactions were like the later ones. Therefore, in the animals used for the experiment there are, at least transient, similar individual differences in sensitization, as in human hosts. Intracardial injections of neoarsplenamine in highly sensitized guinea-pigs have caused a morbilliform exanthem in a number of animals. Anaphylactic symptoms did not appear in connection with these injections. This sensitization was not accomplished in the lower apes and dogs, and in rabbits only in an incomplete degree.

AUTHOR'S SUMMARY.

THE INFLUENCE OF THE BAYER SUBSTANCES ON ANAPHYLACTIC SHOCK. W. A. COLLIER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **52**:191, 1927.

The reinjection of serum to which is added Bayer 205 in animals previously sensitized with normal serum gives no anaphylactic reaction or a weak one. If Bayer 205 is injected into the sensitized animals before reinjection of antigen, the anaphylactic reaction is also inhibited. Precipitin reactions are not influenced by the Bayer substances.

PAUL R. CANNON.

FURTHER EXPERIMENTS ON THE THEORY OF ANTIBODY FORMATION. E. FRIEDBERGER and S. SEIDENBERG, Ztschr. f. Immunitätsforsch. u. exper. Therap. **52**:202, 1927.

The injection intravenously into rabbits of small amounts of killed typhoid bacilli, introduced slowly and over a long period (ninety minutes) by means of a motor driven syringe, leads to smaller amounts of agglutinins than when the same amount is given rapidly. This difference is not noted when large doses of antigen are given. The explanation of this phenomenon rests on Pfeiffer's irritation theory. The slow injection of antigen leads to a fine type of agglutination, whereas the rapid injection leads to a coarse type.

PAUL R. CANNON.

INFLUENCE OF THE PERIOD OF A SINGLE INJECTION OF ANTIGEN ON ANAPHYLAXIS IN GUINEA-PIGS. E. FRIEDBERGER and S. SEIDENBERG, Ztschr. f. Immunitätsforsch. u. exper. Therap. **52**:214, 1927.

Guinea-pigs injected with small doses of antigen are made much more hypersensitive when the antigen is given slowly, subcutaneously. The theoretical and practical consequences of this fact are discussed.

PAUL R. CANNON.

THE ACTION OF PATHOGENIC BACTERIA AFTER SLOW INJECTION AND THE THERAPEUTIC VALUE OF SERUM AFTER PROTRACTED INJECTION. E. FRIEDBERGER and T. IKEDA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **52**:221, 1927.

The protracted injection of the El Tor vibrio and typhoid bacilli proved more infectious than a rapid injection. Bacteriolytic serum was more effective therapeutically when injected rapidly.

PAUL R. CANNON.

CONCEPTIONS OF ANTIBODIES AND THE CONSTELLATION OF SERUM PROTEINS. G. KAPSENBERG and T. RISPENS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **52**:227, 1927.

This paper describes and discusses studies as to the relations of agglutinins to the globulins of antiserums. In all cases the total globulin fraction contained all and the albumin fraction none of the agglutinin. The quantitative investigations showed that equal quantities of pseudoglobulin, euglobulin or total globulin contained the same quantities of agglutinin. Differences were not found between rabbit and horse serum. The authors then discussed a theory concerning the constellation of serum proteins and the relation of antibodies to serum fractions in general.

PAUL R. CANNON.

THE ANTIGENIC PROPERTIES OF ANATOXIN. M. ISABOLINSKY and W. GITOWITSCH, Ztschr. f. Immunitätsforsch. u. exper. Therap. **52**:334, 1927.

The authors maintain that the combining properties of anatoxin signify more than precipitability. Anatoxin retained its immunizing property for one year. For the preparation of anatoxin it is best to use an old toxin which possesses a good combining power, a more important factor than toxicity. The use of 5 per cent formaldehyde in the production of anatoxin is advantageous in that the vessel containing the mixture can be frequently opened without danger of bacterial contamination.

PAUL R. CANNON.

THE HETEROGENETIC COMPLEMENT FIXING ANTIBODIES. J. L. KRITSCHEWSKI and R. E. MESSIK, Ztschr. f. Immunitätsforsch. u. exper. Therap. **52**:339, 1927.

The heterogenetic antigen of sheep, hen, turtle and cat, both from red cells and organs, is soluble in alcohol and produces complement fixing antibodies on injection into animals. The stroma of the red cells contains all the heterogenetic antigen, although some preparations of hemoglobin are active probably because of inadequate methods for the complete separation of stroma from hemoglobin.

PAUL R. CANNON.

THE PRESENCE OF ISO-AGGLUTININS IN HUMAN ORGANS. E. WITEBSKY and K. OKABE, Ztschr. f. Immunitätsforsch. u. exper. Therap. **52**:359, 1927.

In the alcoholic extract of organs of group A are found the same lipoid antigens as in the blood. This lipoid antigen is found not only in normal organs but also in malignant tumors of persons belonging to group A. The antigen is also present in the blood serum, but it must be extracted with alcohol. The brain, however, in spite of its lipoid rich content, does not contain the group A antigen. In the organ extracts of group B, the lipoid specific for this group is found only in the liver. The iso-agglutinins, therefore, represent cell groups instead of blood groups. These observations suggest the possibility of identifying the origin of individuals by means of organ extract reactions.

PAUL R. CANNON.

THE ACTION OF DIPHTHERIA ANTITOXIN IN VITRO AND IN VIVO. BECKER, Ztschr. f. Immunitätsforsch u. exper. Therap. **52**:402, 1927.

Toxic diphtheria bacilli growing in diphtheria antitoxin rapidly become less toxic or lose their toxin forming power entirely. Normal serum injected simultaneously with washed toxic diphtheria bacilli does not affect the infection of guinea-pigs; one-tenth unit of antitoxin hinders the infection slightly, one unit decreases it distinctly and ten units prevents its effects. Antitoxin (ten units) injected twenty hours after infection shortens the course and if given forty hours after the infection is still able to cure. The presence of antitoxin stimulates the auto-immunization of the infected animal.

PAUL R. CANNON.

THE ANTIBACTERIAL AND ANTICOMPLEMENTARY ACTION OF BILE. W. PFANNENSTIEL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **52**:445, 1927.

The combination of pure bile with serum decreases the antibacterial action of both substances for typhoid bacilli and the mixture serves as an excellent enrichment medium. The higher the bactericidal action of the serum, the higher the concentration of bile which must be used to neutralize this action. Bile acid salts alone inhibit the antibacterial action of serum. Urea does not do this. The loss of the antibacterial action of serum is associated with a disturbance of complement function. The action of bile and bile acid salts depends on physical chemical changes which are produced in the colloidal system of proteins and lipoids of the serum.

PAUL R. CANNON.

## Tumors

CYSTIC LYMPHANGIOMA OF THE GREATER OMENTUM. P. M. GRAUSMAN and H. L. JAFFE, Ann. Surg. **77**:66, 1928.

The growth is regarded as a true blastoma arising from undifferentiated mesenchyme.

N. ENZER.

CARCINOMA OF THE OVARY IN INFANCY. V. C. HUNT, Ann. Surg. **77**:84, 1928.

This is a report of a carcinoma of the ovary weighing 1,000 Gm. in an infant, aged 17 months. There was marked precocious sexual development, corresponding to puberty.

N. ENZER.

MALIGNANT DISEASE OF THE THYROID GLAND. J. DE J. PEMBERTON. Ann. Surg. **77**:367, 1928.

In 457 cases of malignant disease of the thyroid, 87 per cent gave definite evidence of a prexisting benign goiter. The tumors were classified as: sarcoma, 1 per cent; diffuse carcinoma, 25 per cent; carcinoma and fetal adenoma, 38 per cent; and papillary adenocarcinoma, 30 per cent. Malignancy should be suspected in all tumors of the thyroid in which there has been recent rapid growth. In only 35 per cent was the condition suspected clinically. Metastases are frequent and may occur early. In order of frequency, the sites of secondary involvement are: regional lymph nodes, 69 per cent; lungs, 24 per cent; chest and mediastinum, 16 per cent, and bones, 6 per cent.

N. ENZER.

FURTHER OBSERVATIONS ON AN EXPERIMENTALLY PRODUCED SARCOMA OF THE CHICKEN. ERNEST STURM and JAMES B. MURPHY, J. Exper. Med. **47**:493, 1928.

Numerous attempts were made to separate from the cells of a tar sarcoma of the chicken a causative agent for the growth. Experiments with filtrates and desiccates injected as such or in combinations with embryonic tissues have all failed to give positive results. So too have injections of filtrates and desiccates into developing chick embryos failed to yield a response. The results confirm those of previous work with the tumor in this laboratory. The growth would appear to differ in a fundamental respect from all tumors of the fowl previously studied.

A CASE OF PRIMARY MULTIPLE CUTANEOUS EPITHELIOMA. M. CAMPLANI, Tumori **2**:282, 1928.

Camplani reports a case of multiple carcinomatous skin lesions with slow development in a woman, aged 64. Some of them have the appearance of rodent ulcers, others those of papillary epithelioma. His final diagnosis is multiple, idiopathic, benign epithelioma of the skin coexistent with, or consecutive to, Bowen's disease.

W. OPHÜLS.

TRAUMA AND MALIGNANT GROWTHS. BLUMENTHAL, F. Med. Klin. **24**:249, 1928.

1. The degree of trauma is not as important as its consequences. Sequelae need not necessarily appear in the form of necrosis as long as there are definite clinical evidences of inflammation and "bridging symptoms."

2. There is no definite time relationship between trauma and malignant manifestations, but a too short interval rather than a long interval speaks against this relationship.

3. Metastases occurring in unusual places associated with trauma can be held as due to the trauma.

4. The malignant growth does not have to arise at the exact spot of the trauma, but in the zone affected by it.

In the ensuing discussion, Dr. Schmieden of Frankfurt A. M. concluded as follows:

1. The tumor must not have been observed before the trauma.

2. The trauma must have been considerable.

3. The site of the trauma and the tumor must be identical.

4. A logical time interval must elapse between the trauma and the appearance of the tumor.

G. RUKSTINAT.

## Medicolegal Pathology

ALCOHOL AND THE MOTORIST. G. CARTER, Brit. M. J. **2**:333, 1927.

The first paper in a discussion of this topic at the meeting of the Section of Forensic Medicine of the British Medical Association in Edinburgh last year was devoted to the experiences the author had had in Sheffield in examining persons arrested for being under the influence of alcohol while driving motor vehicles. In Sheffield, the urine is collected in a room adjacent to the cell, where the prisoner urinates into a urinal in the wall; there is some question, naturally, regarding the validity of the evidence so obtained.

Other phases discussed were allowing the prisoner to have his own physician present at any examination to which he should be subjected, the use of the word "drunk," or the phrase "under the influence" in statutes, the large rôle played by the court in deciding from evidence whether the accused was intoxicated, the usefulness of quantitative tests of the breath for alcohol and the results Carter had obtained with Southgate and Mellanby, his associates in Sheffield.

Some studies are already reported in the *British Medical Journal* of March 13, 1926, and here reference is made to the ratio of 1 to 1.34 for amounts of alcohol in the blood and urine, respectively. It seems from these studies in Sheffield, that when there are amounts of alcohol in the urine equivalent to 300 cu. mm. of alcohol for 200 cc. of urine that the individual is definitely intoxicated. There has occurred in such persons a loss of power of delicate nerve control, so requisite for safe driving. Thirty drops of urine suffices for the chemical work. Another point brought out was that persons go to sleep soon after being locked up for drunkenness.

E. R. LE COUNT.

BARBITAL AND DIALLYL BARBITURIC ACID POISONING. DARGEIN and DORÉ, Bull. et mém. Soc. méd. d. hôp. de Paris **43**:1392, 1927.

Diallyl barbituric acid, a barbituric acid compound with two allyl instead of two ethyl groups, caused one case of poisoning, and this drug and barbital another case. In the latter, a profound coma occurred lasting twenty-four hours and ending in death. An intense engorgement of the leptomeningeal vessels with minute hemorrhages throughout the brain was found. The amounts of the two drugs taken were not learned; both were recovered chemically.

In the case of poisoning by diallyl barbituric acid alone, about 42 grains (2.73 Gm.) were taken in an attempt to commit suicide. Coma lasted seventy-eight

hours with some convulsions and opisthotonos, trismus, and tonic spasm of the sternomastoid muscles which projected the head ventrally. Emptying of the bowel and bladder were inhibited. The pulse and respiration were undisturbed and there was no dyspnea. Recovery occurred in spite of the fact that the toxicity of diallyl barbituric acid is twice that of barbital.

E. R. Le Count.

Barbituric Trophic Gangrene. H. Claude, A. Lamache and H. Daussy, L'Encephale **22**:439, 1927.

In a thesis in 1924, Andre Tardieu described the characteristics of poisoning by barbital as varying intensities of coma, confusional excitation with oneirogmus, muttering delirium, transient paralyses, paretospasmodic dysarthria, subacute and transitory paralysis of the external orbital muscles, liver and renal inadequacy, a polymorphous pruritic exanthem and prolonged convalescence with coma. To such a list of symptoms caused by the barbituric acid drugs, Claude and his associates add polyneuritis with patches of dry gangrene of the sole, heel and tips of some toes of one foot.

Three attempts at suicide with barbital were made by a woman 35 years of age, and following the first attempt when only 3 Gm. were taken, there were some symptoms of polyneuritis. Regarding what followed the second attempt with 7.5 Gm., she had few recollections. At the third effort, a huge dose of 15 Gm. was taken, and with it 5 Gm. of phenobarbital. The spots of gangrene appeared ten days later; recovery occurred after about a month.

E. R. Le Count.

Birth Injury of the Brain. W. Cohen, Zentralbl. f. Gynäk. **51**:505, 1927.

During labor at term in a primipara, which was prolonged over several hours, a marked irregularity in fetal heart beats was noted before the head was engaged or the waters had broken. The labor pains were severe. The child was well developed, but had cyanosis and convulsions and died after thirty-four hours. Grossly, there were a number of places in the white substance of the cerebrum which were slightly yellow, and by microscopic examination this color was found to be due to fatty degeneration of the neuroglia. The opinion is expressed that the focal fatty change was caused by the bruising, which the head received when jammed against the maternal bony pelvis by the uterine contractions. Hemorrhages were altogether absent.

E. R. Le Count.

Eclampsia Without Convulsion. H. H. Schmid, Zentralbl. f. Gynäk. **51**: 913 and 1535, 1927.

At a medicolegal postmortem examination, because death during the sixth month of pregnancy was unexpected, a considerable intraventricular hemorrhage of the brain was found with the necroses in the liver characteristic of eclampsia. Previous to coma there was nausea with somnolence and vomiting, but no convulsions. Anasarca and the usual urine alterations were also present. Apoplexy with eclampsia is not uncommon, but eclampsia without convulsions is rare.

E. R. Le Count.

Infanticide. G. Strassmann, Zentralbl. f. Gynäk. **51**:2047, 1928.

At a meeting of gynecologists in Breslau, Strassmann told some of his experiences and exhibited preparations illustrating the difficulties of establishing murder of new-born infants. These pertained, among others, to disappearance of air from the lungs after death, floating of decomposed lungs, the wide range in the amount of air in the stomach and bowel, the improbability of extensive cranial fractures and fractures of the cranial base by precipitate birth with the mother standing, births in water closets, the estimation of the duration of life, tentorium tears, intracranial hemorrhages and detection of the manner of birth. He emphasized the absence of the usual signs of asphyxia, caused by smothering with soft coverings.

E. R. Le Count.

## Technical

COLORIMETRIC DEMONSTRATION OF IRON AND HEMOGLOBIN IN BLOOD. S. Y. WONG, J. Biol. Chem. **77**:409, 1928.

A method is suggested whereby the iron content may be separated from a blood or hemoglobin preparation and estimated colorimetrically as ferric sulphocyanate.

ARTHUR LOCKE.

A NEW BLOOD SUGAR METHOD. O. FOLIN, J. Biol. Chem. **77**:421, 1928.

A new method is proposed wherein the sugar is oxidized with alkaline potassium ferricyanide, and the resulting ferrocyanide measured colorimetrically as Prussian blue. The increased delicacy of the new method is such that duplicate determinations can be made on the extract from 0.1 cc. of normal blood.

ARTHUR LOCKE.

CHOLESTEROL DETERMINATION IN BLOOD. D. ACÉL, Deutsche med. Wchnschr. **54**:431, 1928.

Acél describes a new method for the determination of cholesterol in the blood which is shorter, easier, more accurate, and just as satisfactory theoretically and practically as the older methods. It consists in saponification with lye, subsequent solution in chloroform and the addition of sulphuric acid at the time the characteristic color appears. This is then compared colorimetrically with a standard containing a known quantity of cholesterol, and from which the milligram per hundred cubic centimeters of cholesterol in the tested sample can be calculated. As little as 0.5 cc. of blood can be used satisfactorily. The method gives from 5 to 7 per cent more cholesterol than the Autenrieth-Funk method, which the author believes is due to the greater loss from the procedure employed in the latter.

THE STAINING OF URINE SEDIMENT WITH SEYDERHELM'S SOLUTION. GEORGE KEMENY, Klin. Wchnschr. **7**:694, 1928.

A drop of Seyderhelm's solution (a mixture of congo red and trypan blue) is added to a drop of urine sediment on a slide and the mixture covered with a cover glass. Living cells are unstained, injured cells stain faintly, and necrotic cells stain intensively from a dark blue to a dark red in different shades. The results of Kemeny's studies have demonstrated that elements of living tissue occur chiefly in acute diseases of the urinary tract, while the dead cells appear in the sediment with subacute or chronic diseases.

E. F. HIRSCH.

THE CHOLESTEROL-FAT TEST IN CIRRHOSIS OF THE LIVER. A. W. ELMER and M. SCHEPS, Klin. Wchnschr. **7**:1083, 1928.

As the cholesterol-fat test is positive in many normal persons as well as in many with disease of the liver, it cannot be regarded as dependable in the diagnosis of cirrhosis of the liver, and as a liver function test it offers much less information than the established methods with galactose, levulose, or tetrachlor phenolphthalein.

AUTHORS' SUMMARY (IN PART).

COPPER PEROXIDASE REACTION OF BLOOD. K. SHOJI, Tohoku J. Exper. Med. **10**:293, 299 and 305, 1928.

In his first paper Shoji discusses the application to animals of the copper peroxidase reaction for determining the myeloid elements of the blood. In adapting the original method of Sato and Sekiyo to the blood of rabbits he uses three reagents: solution A′—copper sulphate, 0.5 per cent; solution B′—0.2 Gm. of benzidine rubbed with a few drops of water and added to 200 cc. of water and

filtered; to the filtrate, 8 drops of hydrogen peroxide (3 per cent) is added; solution C′—either (*a*) safranine, (*b*) neutral red or (*c*) carbol fuchsin. This differs from those used in human blood only in that there is twice as much hydrogen peroxide in solution B′. Using a fresh dried blood smear, solution A′ is applied, most of it is poured off, and solution B′ is applied. After two minutes it is washed with water and counterstained with solution C′. By this method, the granules of the myeloid leukocytes are stained a deep bluish green. Shoji concludes that of all the animals the blood of which he tried there were none whose myeloid elements were negative to peroxidase.

In his second paper, a technic is described for the differentiation of the myeloid and lymphatic leukocytes of animal blood in a counting chamber. Here, likewise, three specially prepared reagents are used and an exact method of preparing the fluid must obtain. By this method, the myeloid leukocytes are stained dark greenish-blue, the monocytes appear faintly red with blue granules, and the lymphocytes distinctly red without any shade of blue or green. By a slight modification of this method a differentiation between the true eosinophil and the pseudo-eosinophil may be made.

The last paper dealt with a rapid method of differentiation of the lymphatic and myeloid leukocytes in the spinal fluid. Two solutions are used: solution A—rub 2 Gm. of purified benzidine in a mortar with a small amount of a mixture of equal quantities of water and glycerin; add slowly until the amount reaches 100 cc.; filter and add three drops of 3 per cent hydrogen peroxide to the filtrate; solution B— 3 per cent copper sulphate solution, 300 cc.; 1 per cent aqueous safranine solution, 9 cc.; 30 per cent acetic acid, 1 cc. Solution A is drawn into a leukocyte pipet to the mark 0.9 and solution B to 1.0. The pipet is then quickly filled with spinal fluid to the mark 11 and thoroughly mixed. By this method the myeloid leukocytes appear blue and the lymphocytes intensely red.

EDWIN P. JORDAN.

# Society Transactions

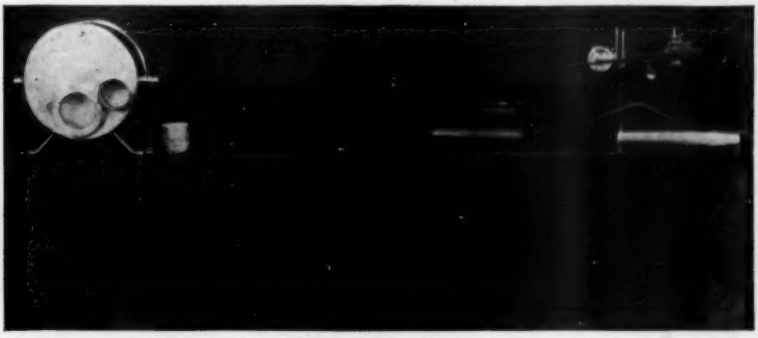
## CHICAGO PATHOLOGICAL SOCIETY

*Regular Monthly Meeting, May 14, 1928*

OSCAR T. SCHULTZ, *President pro tempore, in the Chair*

THE EFFICIENCY OF WORK DURING EXPERIMENTAL HYPERTHYROIDISM. GEORGE M. CURTIS.

It is now well recognized that hyperthyroidism, whether produced experimentally or observed clinically in patients with goiter, often results in an enormous increase in oxygen consumption and associated energy output. Likewise, patients with hyperthyroidism fatigue rather easily following muscular exertion. Boothby (*J. A. M. A.* **81**:795 [Sept. 8] 1923) pointed out that if they are up and about, the consumption of food may even exceed that of a soldier in active duty, amounting almost to a "bulimia." The loss of weight occurring in these patients on ordinary diets is a commonplace observation. The increased consumption of oxygen and



Apparatus for determination of the work metabolism in small mammals.

output of energy during muscular work in normal persons is self-evident, as well as the higher ration of the active soldier.

In view of these conditions, it was of interest to my associates and me to study the work economy in experimentally produced hyperthyroidism, in a search for data bearing on the cause of the increased consumption of food, or the loss of weight in active patients with hyperthyroidism when maintained on ordinary diets. It is a matter of interest that the experimental results obtained reveal that in experimental hyperthyroidism, work is done much less economically than under normal conditions.

Young male rats were used for the experiments. During the study they were maintained on a bread and milk diet. The work done was essentially the running of approximately a kilometer in an hour, in an especially devised revolving metal drum. The gas exchange was determined after the Haldane (*J. Physiol.* **13**:419, 1892) principle, in a closed circuit apparatus and gravimetrically. The apparatus was originally devised by Asher (Curtis: *Biochem. Ztschr.* **164**:97, 1925) and was subsequently modified according to the needs of extensive use. The last model of the apparatus is presented in the accompanying illustration. Some of the basal determinations were made in the work chamber, with the animal at rest, but the majority were made in a small, especially constructed glass chamber modelled after the device used by Asher and Danoff (*Biochem. Ztschr.* **93**:44, 1919).

Animals were first accustomed to the rest apparatus, after which a series of determinations of basal metabolism in the postabsorptive state was made. They were then trained to run in the revolving drum, following which a series of basal metabolic determinations during that type of work was made. The animals were then fed by stomach tube 35 centigrams of thyroid extract each day; beginning on the fifth day, determinations of the basal metabolic rate were again made. Alternating with these, a series of determinations of work in the basal metabolic state was made.

Four series of determinations of the gas exchange were thus made on each of the animals studied, all during the basal metabolic state—the normal, after thyroid was fed, during work and during work after thyroid was fed. The results thus obtained (Curtis: *Biochem. Ztschr.* **167**:321, 1926) reveal that in experimental hyperthyroidism the rats work much less economically than normally. To do the same amount of work, there is an increase in oxygen consumption of more than 50 per cent, with a corresponding increase in energy output. An experimental basis is thus afforded to account for a part of the loss of weight and excess intake of food in active patients with hyperthyroidism. This feature was first pointed out by Plummer and Boothby (*Am. J. Physiol.* **63**:406, 1923) in patients with hyperthyroidism. This work is offered as an experimental confirmation, since the symptoms are similar. The phenomenon has more recently been rediscovered by Kisch (*Klin. Wchnschr.* **5**:697, 1926). It costs the patient with hyperthyroidism many more calories to climb a given flight of stairs than a normal person of the same size and weight. Likewise, animals with experimental hyperthyroidism consume much more oxygen in running approximately a kilometer in an hour than do the same animals under normal conditions.

DISCUSSION

E. Pribram: With Zuntz in 1907, I studied the metabolism of dogs after exercise. During the rest period, the metabolic rate was higher than normal. If work was one while the animal was exhausted, the rate was the same as has been found in rats with experimentally produced hyperthyroidism. In the experiments reported, the respiratory quotient is high; in our experiments, it was as low as 0.6. It would be interesting to know what results would occur in the metabolism and the respiratory quotient in these animals under similar conditions of rest and work.

Frank Smithies: Were any experiments made with thyroxin? Was the thyroid gland intoxication carried to the extent where the animals had diarrhea, or electrical changes of the muscles?

George M. Curtis: I discussed with Loewe, also of Zuntz's laboratory, the results presented here. He seemed to think that the higher respiratory quotient is caused by an initial washing out of the carbon dioxide in the tissues, and that if the experiments were carried beyond this interval, the respiratory quotient would be low. Burroughs Wellcome and Company thyroid gland preparations were used but no thyroxin. Enough was given to cause diarrhea and symptoms so severe that the animals died. With a basal metabolic rate of plus 40 the animals are exhausted. In repeating these experiments, it is better to give one large dose followed by small doses.

Air Embolism of the Pulmonary Circulation. George Rukstinat and E. R. Le Count.

A study of experimental embolism of air in the pulmonary veins and systemic arteries in guinea-pigs was reported, together with clinical applications of the results obtained and a review of the literature and different views which are held concerning the manner in which death results from this kind of air embolism.

The Absence of Seasonal Change in the Toxicogenicity of the Diphtheria Bacillus. Arthur Locke and E. R. Main.

A good quality of diphtheria toxin can be prepared as readily during the summer months as during the winter season. If variations in the composition of the broth

are avoided, the toxin production in Lf units on a given type of medium and by a given strain of organisms may be so controlled as to be practically constant throughout the year.

Variations in toxicity, degree of antigenicity and antitoxin-binding avidity may be detected through variations in unit flocculation time. A significant variation could not be found in the unit flocculation time of the toxins produced by different strains of the diphtheria bacillus, on mediums of varied composition, during different seasons or during different stages of the period of active growth.

FLUCTUATION IN THE HYDROGEN ION CONCENTRATION OF SALIVA IN EPILEPSY. A. M. SAUNDERS.

Epilepsy has been considered an acidosis, and the treatment consisted formerly in the restriction of meats, vegetables being substituted for them to increase the alkalinity of the system. Bisgaard and Norvig were the first to demonstrate that rather the opposite—an alkalosis—was the underlying condition in epilepsy. They demonstrated the retention of basic substances in blood serum and urine. This dysregulation, as it was called, was attributed to a disturbed function of the parathyroid gland. I had an opportunity to visit St. Hans Hospital in Denmark at the time this work was being done. Norvig did not obtain results with the commercial parathyroid extract in the treatment of persons with epilepsy, but with the use of freshly prepared extracts of the gland in normal salt, he succeeded in cutting down the number of epileptic seizures and in decreasing the irritability and dulness of the patients under treatment. I have not heard of their work recently, but many other authors since then have substantiated the observations of alkalosis in epilepsy. The diet now given to epileptic patients has been changed to a ketogenic diet consisting of high fat and protein ratios. Excellent results, producing a cure in many, have been obtained.

In their investigation of the reaction of blood serums, Marrack and Gilbert were unable to demonstrate definite changes in the reaction of the blood of epileptic persons. There are many conflicting symptoms in this work, and it occurred to me that an investigation of the reaction of some of the secretions of the body that could be obtained easily at frequent intervals and without discomfort to the patient might be of interest. The latter factor is an important criterion in working with patients in state hospitals.

The hydrogen ion concentration of the saliva was estimated by the La Motte colorimetric method. For this study, patients were selected who had an epileptic seizure only rarely, for instance, one a month, and others who were subject to frequent seizures, about one or more a day.

The patients had a light breakfast in the morning, after which they cleaned their teeth carefully with a tooth brush and water under the supervision of a trained nurse. They were also instructed to gargle and rinse the mouth repeatedly with water. After that, they were kept in the examining room under close observation. They did not receive food nor did they put anything else into their mouth during the five or six hours of examination. Every fifteen minutes, the nurse had the patient expectorate into a large receptacle to remove any saliva that might have accumulated and been held in the mouth. Then a second small container, which had been cleaned and rinsed in distilled water, was given the patient, and he was instructed to draw the saliva up from underneath his tongue. The specimens thus obtained were immediately tested and the results recorded.

The La Motte determination requires only a few minutes. We consider this important, because the possibility for a change in reaction due to bacterial activity is reduced to a minimum. Patients with frequent convulsions and also those few who had convulsions while under our direct observation showed a marked fluctuation in the reaction. Other patients who had not had convulsions for the last few days or for several days following showed slight if any fluctuation, giving a curve like that of the normal controls. The fluctuation in the reaction seemed to have a definite relationship to the convulsive period.

In the normal controls as well as in patients having convulsions rarely, the reaction varied only between 6.7 and 7 during five or six hours. In the epileptic patients who had convulsions during the experiment, a $p_H$ reaction varying from 7.1 to 5.8, which changed abruptly, was found. Immediately or soon after a convulsion, there was a change to the more acid reaction. The complete report is published in the *Journal of the American Medical Association* (**91**:244 [July 28] 1928).

DISCUSSION

FRANK SMITHIES: Were experiments made with bacteria-free saliva? There are many normal variations in the reaction of saliva.

E. PRIBRAM: It seems to me necessary to check the bacterial content during the experiment, and also to find out what substances are causing the acid and alkaline fluctuations.

A. M. SAUNDERS: The bacterial content was not controlled.

BACTERIOLOGIC STUDIES IN LYMPHOGRANULOMATOSIS. H. C. SWEANY.

Bacteriologic studies on material from five patients with lymphogranuloma are reported: Four of the tissues examined were from the retroperitoneal glands and one, from the glands of the hilum of the lung.

A child, aged 8 years, had been pronounced tuberculous because of a biopsy examination one and a half years previously. Another patient, a woman, aged 35, manifested symptoms of Addison's disease with many foci in the skin resembling abscesses. A man, aged 28, with a mediastinal involvement, had a cervical lymph node removed. It caused an infection in the knee joint of a guinea-pig, from which diphtheroids and acid-fast bacilli were recovered. Bacteria were not found by direct examination. This suggests more that the methods are at fault, perhaps, than any lack of infection, and is comparable to finding organisms in fibrotic tuberculous glands in cattle. One patient did not reveal any acid-fast bacilli either directly or on passage. Only a pleomorphic diphtheroid was found that may have been the one so frequently described.

The last patient was a woman, aged 60, from whom a cervical gland had been removed five years before and pronounced tuberculous. A typical "abdominal type" of Hodgkin's disease was present. There were many caseous foci in the fibrotic parts of the glands and spleen. Abscesses were found in the lungs in which there were almost pure cultures of acid-fast bacilli. In the caseous foci in the spleen were found small knots of organisms that possessed a natural yellow color and resembled a granular tubercle bacillus in morphology. They seemed to originate from granules and gradually to expand into colonies. From the caseated material and from the abscesses in the lungs, a variegated flora was obtained by cultures. One organism looked and reacted much like an unstable human type of tubercle bacillus. Another looked like an avian type but reacted like a human type of low virulence. A third was a pleomorphic diphtheroid. Perhaps others also were present. This pleomorphic form was found chiefly in the newly formed glandular enlargements. Acid-fast forms were found only in the caseous tissues or abscesses. After growth on glycerin agar, both acid-fast forms lost part of the acid fastness, especially immediately after their isolation. Later, both seem to have become stabilized until they are now continually acid fast.

There is good evidence to show that lymphogranuloma is the result of a parasitic infection in a particular type of host. Perhaps it is a combination of the two factors. The facts that the process runs a typical course of an infectious disease; that the pathologic changes are typical, and that almost always a pleomorphic bacterial flora is present seem to remove much doubt about its infectiousness. It is rarely contagious, first because the host must be prepared for the symbiotic existence of a parasite that will ordinarily not reproduce the pathologic changes in an unprepared host; secondly, the disease is rare, and, thirdly, it runs its course almost entirely enclosed within glands. The fact that a type cell that metastasizes bodily, such as is found in malignant conditions, is not present seems to remove

much doubt as to its malignancy. The mononuclear and multinuclear giant cells are transplants from bone marrow and appear only after the acute inflammatory lymphoid hyperplasia has passed. Many times these giant cells can be found only with difficulty. The fibrosis seems to be an attempt at healing and reveals the degree of chronicity.

The relationship to tuberculosis is suggested because of the frequent presence of acid-fast bacilli that resemble a degenerating type of organism. It is possible that a degenerated form of tubercle bacillus is the origin of the process. This degenerate perhaps passes over into a diphtheroid type that is practically a distinct species and may be so considered. Experimentally, I have produced variations of rare strains of tubercle bacilli that resemble such a change. In one guinea-pig, the multinuclear giant cells such as are found in persons with Hodgkin's disease were obtained from a Berkefeld filtrate of tuberculous materials.

On the other hand, the possibility of secondary infection with variegated acid-fast and diphtheroid bacilli must not be entirely overlooked until more experimental proof is produced. It seems, however, that it is more than a coincidence that such cycles of organisms are so frequently found when other conditions like carcinoma rarely ever yield them. The universal presence of tubercle bacilli in the human body; the fact that tubercle bacilli undergo degenerative changes occasionally, which resemble such forms found, and the fact that there are transition forms of glandular tuberculosis that seem to shade off into a Hodgkin's-like disease are arguments in favor of a tuberculosis-like origin. There is also an ubiquity of diphtheroids, but the frequent presence of acid-fast forms discounts this origin. The whole picture appears more like a heterogeneous degeneration of an atypical strain of tubercle bacilli in a particular type of host, that has failed to overcome the parasite. This seems to be the most logical explanation from available facts.

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## LOS ANGELES PATHOLOGICAL SOCIETY

*Regular Meeting, June 12, 1928*

NEWTON EVANS, *Presiding*

FECALITHS IN ACUTE APPENDICITIS. LAWRENCE PARSONS and HOWARD A. BALL.

In a review of the cases of acute appendicitis since 1925, in which pathologic examinations have been made at the Los Angeles County General Hospital, we have recorded some observations as to the occurrence of fecaliths, and calculated their incidence in a series of 558 cases.

The percentage for the different years varies from 22.4 to 31.5, with an average of 26 per cent for the entire series. These figures are about 10 per cent lower than the figures given by Deaver in his monograph. This variation in figures may be due to the fact that fecaliths have not been particularly looked for until recently, and consequently not described even though present. Unquestionably many fecaliths are lost in ruptured appendixes and by manipulation of operation are pressed back into the cecum, or, as van Zwalenburg believes, from the intra-appendiceal pressure alone.

We believe that a fecalith is any bolus of fecal material, inspissated to the extent that it cannot readily be molded under pressure; that is, that it has a fixed shape which it maintains, and does not necessarily have to contain calcareous material or present lamination.

It was noted in many of the specimens containing fecaliths, that the lumen beyond the site of the fecalith was distended with stercoraceous pus, and not a few were perforated. In some cases, identical conditions were found when a stenosis of the lumen existed near the base. This agrees well with van Zwalen-

burg's conception of the hydromechanics involved. (One should also consult Dieulafoy: Text-Book of Medicine, chapter on appendicitis).

It is our belief that with an incidence as high as Deaver's figures, or our own, an etiologic relationship must exist, and that one step in this etiology is the occlusion of the lumen, following which the contents of the appendix cannot empty into the cecum, the material then furnishing a good soil for bacterial growth.

May not some pathologic physiologic condition concerning the muscular mechanism of the appendix be a factor in the formation of fecaliths?

INTESTINAL PROTOZOA. JOHN F. KESSEL.

The laboratory department of Los Angeles General Hospital for the parasitologic examination of fecal specimens was established on Feb. 14, 1928, with a trained parasitologist in charge. A summary of the results of the first three months' work has been made. During that period, 1,085 examinations for protozoa were made on 478 patients, which gives an average of 2.2 examinations per patient. Of this number, 32.2 per cent of the patients gave positive cultures for one or more species of intestinal protozoa. Had a routine examination been made and six stools per patient examined, the incidence would probably be increased about 25 per cent. The detailed observations are as follows: *Amoeba dysenteriae,* 35, or 7.3 per cent; *Entameba coli,* 66, or 14.0 per cent; *Entameba nana,* 59, or 12.3 per cent; *Iodamoeba,* 7, or 1.5 per cent; *Councilmania,* 11, or 2.3 per cent; *Giardia,* 11, or 2.3 per cent; *Chilomastix,* 32, or 6.9 per cent, and *Trichomonas,* 21, or 4.4 per cent.

While routine protozoologic examinations have not been undertaken in connection with the autopsies, I did come in contact with one interesting autopsy at which a pronounced ulcerative colitis was found. The patient had been in the hospital two days and had died without any specimen of fecal material having been sent to the laboratory. At autopsy, the pathologist found some ulcers which he thought at first might be carcinoma, but on investigation they proved to be benign ulcers. In one of these, which was in the region of the rectum, amebas were found. In order to find amebas, I had to search over ten or twelve slides. Amebas were not found in the cecum, which showed a great deal of grayish mucus, but there were great numbers of *Trichomonas* in this mucus. This mucus was characteristic of infection from *Trichomonas,* similar to that which I found in experimental kittens. However, before I could fix the tissue, it had been washed and much of the mucus removed. While I found the same characteristic diphtheritic colitis in the region of the cecum which I found in the experimental kittens, I have not found any *Trichomonas* in the tissue as I did in that of the kittens. Wenyon, in his textbook, illustrates the invasion of human tissue by *Trichomonas,* this being the only case on record as far as I know. The question of the pathogenicity of *Trichomonas* is not answered, and I present this merely in order to call attention to a problem which I think is worth investigating.

It was also found that the exudate from fifteen cases indicated a bacillary dysentery. Of this number, ten were proved to be positive by bacteriologic culture. Manson-Bahr was the first, I believe, to point out the fact that bacillary dysentery infections show a characteristic type of cellular exudate in the stool. During the war, there were a number of men who worked on the subject and who used that method in differentiating between bacillary and amebic dysentery. Since the war, Haughwout, in the Philippines, and also Callender, have brought to one's attention these previous articles and have added from their own experience the fact that cytodiagnosis in bacillary dysentery is important. In the bacillary type of dysentery, the exudate contains a preponderance of polymorphonuclears, together with a large number of macrophages, while in the discharges of amebic dysentery the proportion of polymorphonuclears is small. It is important because one can make a diagnosis in a short time without waiting for the results of the bacteriologic examination, which may require forty-eight hours, sometimes longer,

for an exact determination of the species of the bacillus. When I was in Peking I found that about 90 per cent of the cases of dysentery that came under my observation were bacillary, and I endeavored to employ this method of differentiation and met with a certain amount of success. Checked by bacteriologic culture, I found that 90 per cent of the cases that I thought were bacillary dysentery actually proved to be of this type.

When I began my work here I noticed several stools which indicated a bacillary infection. One of the cases went to autopsy, and the tissue showed a typical picture of bacillary dysentery.

Rounded, moribund amebas and round macrophages are difficult to differentiate in fresh smears, and have been the cause, I think, of a great many false diagnoses. I think a great many so-called epidemics of amebic dysentery were epidemics of bacillary dysentery.

MIXED TUMOR OF THE KIDNEY. RALPH M. CRUMRINE.

I shall report the case of a girl, aged 5, who came into the hospital on June 5 with a complaint of a large mass in the abdomen. She was of poor Mexican parents, and had apparently had little medical attention. According to the history, the mass was first noticed four months before admission, and was described as being the size of a golf ball in the left upper quadrant. When she entered the hospital, she presented an enormous mass in the left side of the abdomen. Emaciation was extreme. The intern described the mass thus: "Abdomen is greatly distended by an enormous mass which causes knob-like, uneven protrusions, the largest of which is in the upper left quadrant below the costal margin, and which bears the mark of a recent puncture point. The mass extends to the right of the umbilicus and down half way between the umbilicus and the symphysis. It is rather firm with areas of somewhat softer consistency, and moves with respiration."

A physician had punctured the abdomen, considering the contents ascites, and secured some bloody fluid. The patient had a red count of 3,200,000 and a white count of 10,400. Urinalysis was negative. When she was sent to the hospital, one outside physician's impressions were that the diagnosis was: (1) sarcoma of kidney; (2) splenomegaly, and (3) megalocolon. Of the visiting physicians who saw her here, the first one said "cancer" and advised consultation with a surgeon immediately. Another said, "I believe the child most probably has a sarcomatosis, left lobe of liver as point of origin. There is a slight possibility that there may be a polycystic kidney."

She died on June 9, and autopsy brought out the following: The abdominal cavity did not contain fluid. A cystic mass was found in the left side which was entirely retroperitoneal in origin, the peritoneum being stretched over it, together with a section of the large intestine. It was roughly the shape of a kidney. On section, it was evidently malignant and contained areas of degeneration. The spleen and liver were not involved. Metastases were not found. The only other point of pathologic interest was bronchopneumonia, not suggestive of any malignant metastases. When removed, the mass weighed 3,180 Gm. Ewing mentions a similar tumor weighing 3,580 Gm. The tumor in this case, was, roughly, the size and shape of a football.

When the tumor was incised, renal tissue could not be made out by gross examination, but microscopic sections showed considerable areas of kidney tissue which was fairly normal except that it was compressed. Further slides showed much infiltration with small carcinoma cells in dense areas and at times in definite adenomatous arrangement. The origin of these cells was not clear; they did not resemble renal elements nor suprarenal tissue. The other principal element was of connective tissue type and appeared sarcomatous. Striated muscle or cartilage was not found, and van Gieson's stain gave little evidence of smooth muscle. One slide showed the left suprarenal as normal tissue surrounded by tumor tissue, but not in itself involved. The right suprarenal and pancreas were normal. The lung showed marked purulent pneumonia and bronchitis, but no tumor.

This tumor was considered to be of the type described by Ewing as "embryonal adenomyosarcoma." Perhaps one should say in this case, "embryonal adenosarcoma," as muscle elements were not found.

RUPTURED TUBAL PREGNANCY PRODUCING FATAL INTESTINAL HEMORRHAGE. LAWRENCE PARSONS.

The case I shall report is the only one of its kind I have ever seen or have been able to find recorded in the standard monographs and textbooks dealing with gynecologic conditions.

Mrs. C. C., aged 25, was admitted to the Los Angeles General Hospital the latter part of May, 1928, about 9 a. m., and died about 2 p. m. the same day. Little history could be elicited, and that only through an interpreter. She had been bleeding fresh blood through the rectum in a considerable quantity for five days. Except for pain in the rectum, the history did not contain any positive information.

She was almost moribund on admission to the hospital, having a weak, thready pulse, and she was bleeding profusely from the rectum. Some were of the opinion that she had acute bacillary dysentery. One clinician believed, however, that the condition was hemorrhage from the bowel, of unknown origin. Proctoscopic examination revealed a normal appearing rectum, but with blood welling up from a point higher than the proctoscope would show. Bimanual examination revealed a mass in the right adnexal region, apparently connected with the uterus.

At autopsy, I found the right uterine tube distended in the middle third to a diameter of 6 cm., with blood clot and spongy tissue, in the interior of which was a small cavity lined with a glistening membrane characteristic of tubal pregnancy. An embryo was not found. The tubal pregnancy had ruptured into the right broad ligament, producing a hematoma 12 cm. in diameter. Adherent to the posterior aspect of this hematoma was a loop of the sigmoid colon. Into the lumen of the bowel at this point, a perforation — in fact, two — 5 mm. in diameter, had occurred. An early generalized peritonitis was present, also considerable exsanguination of the various viscera.

Sections through the tube showed definite chorionic villi, establishing the diagnosis of tubal pregnancy. Sections through the point of rupture into the sigmoid showed only necrosis, apparently from pressure. Placental elements were not found in the muscular wall.

ENAMEL ORGAN TUMORS OF THE MAXILLA (TWO CASES). NEWTON EVANS.

I am presenting two cases of tumor of the jaw occurring in connection with the teeth. Each of the tumors was of the lower jaw. One was of larger size, weighing 800 Gm.; the other was small, about 4 Gm. in weight, and was removed by the hospital dentist.

The two tumors, from a genetic point of view, should be classed together, originating in aberrations of the enamel organs. The mechanism concerned in the beginnings of such tumors was first elucidated by Mallassez, in France, in 1885. Previous to the publication of his work, ideas were confused regarding the nature and origin of these maxillary tumors. Mallassez studied the structure of the tumors and investigated the embryologic changes concerned in the development of the teeth. He found that in the earliest formation of the enamel organs, which consist of an invagination of the surface epithelium, epithelial structures are separated from the surface epithelium along the line of the alveolar process which constitute the anlage of the enamel of the teeth. In association with these successfully functioning organs, there are also found numerous small abortive epithelial groups, which in the embryo and in later life lie in close proximity to the teeth, small masses being found at various levels along the margins of the teeth. To these small, nonfunctioning groups of epithelium Mallassez gave the name of "paradental epithelial débris." These epithelial rests would appear to

be the structures which on occasion display abnormal growth activity and become the starting points of the paradental tumors.

CASE 1.—Perhaps the commonest and most important tumors of such origin are the so-called "adamantinomas." These have a characteristic histologic structure, composed largely of epithelial cell groups, the central cells of which are large and hydropic and sometimes contain calcareous material, while the peripheral cells of the groups are large, elongated and set in a striking radial arrangement. It is evident that this tumor, weighing 800 Gm., and located in the mandible, belongs to this group. A striking histologic characteristic of this particular example was the accumulation in the epithelial cells of the characteristic groups of rounded cell inclusions having the appearance of hyaline material, which with the progress of the growth completely filled the cells, with the final disappearance of the cell structures.

Grossly, the specimen was a large tumor mass including a portion of the mandible with several teeth. The tumor was nodular and contained bony and soft tissues. One cystic cavity was present, measuring 3 cm. in diameter, surrounded by bony tissue and containing thin fluid and sanguineous material. A separate mass of tissue contained two lymph nodes, one of which was as large as a lima bean. Microscopically, these were free from metastatic tumor tissue. The clinical diagnosis preceding the operative removal of the tumor was "carcinoma of the thyroid."

The adamantinomas are essentially carcinomatous in nature, but, as a rule, do not produce metastases.

CASE 2.—The specimen consisted of tissue removed at the dental clinic from the lower jaw of a man, aged 56. Clinically, it was called a "dental cyst." It was situated in close proximity to the first, second and third molars. The tissue was in two fragments, aggregating about 4 cc. in volume, and was grayish white. Microscopically, it consisted of much dense fibrous tissue containing many fibroblasts and groups of plasma cells. The free surface tissues in certain areas consisted of typical granulation tissue, but the greater portion of the surface tissue consisted of stratified squamous epithelial tissue arranged in a remarkably complicated papillary arrangement, and much of it was in a state of disintegration and filled with wandering cells, the great majority of which were polymorphonuclears.

This structure corresponded to the structure of the so-called "radiculodental cysts" which, according to Mallassez, are structures derived from the paradental epithelial débris. Purulent inflammatory changes are usual in this type of lesion, as was the case in the present specimen.

A number of forms of tumors of the jaws of dental origin are recognized in addition to the two typical forms illustrated by the characteristic specimens presented at this time.

# Book Reviews

HISTORICAL SURVEY OF RESEARCHES ON THE STREPTOCOCCI (WITH SPECIAL REFERENCE TO THE IMPORTANCE OF DIFFERENTIAL MEDIA AND MICROPHOTOGRAPHY AS AN AID TO THEIR CLASSIFICATION AND IDENTIFICATION). By D. THOMSON and R. THOMSON. Price, $10. Pp. 430, with 57 plates. Annals of the Pickett-Thomson Research Laboratory, vol. 3, November, 1927.

This ambitious volume takes up the streptococcus group in general and in particular. Volume IV of the *Annals*, however, to be published this year, will deal only with the pathogenic streptococci and the diseases believed to be caused by them. The present work is largely devoted to a long review of the literature on streptococci, their morphology, cultivation, biochemical reactions, soluble products, vitality, virulence and immunity reactions and a historical review of the various streptococci described and named in the past. The review of the attempts to classify streptococci takes seventy pages and contains an article by H. Warren Crowe on the differentiation and classification of the nonhemolytic streptococci. It also contains the authors' attempts at differentiation of streptococci by the aid of photomicrography. This original material covers the last twenty-nine pages and includes the illustrative plates, which are particularly good.

The review of the extensive literature on streptococci makes this volume a convenient and valuable reference work, but it must be noted that much of the material is scattered and that there are numerous important omissions. It is extremely unsatisfactory to find, for example, that the review of "The Attempts to Classify and Differentiate between the Various Kinds of Streptococci" covers the subject only up to the year 1925, although the book was published in November, and the preface is dated May, 1927.

The discussion of morphology is limited practically to the length of the chains, and the interesting question of flattened cocci as a morphologic feature is dismissed by saying, "More rarely the cocci are flattened (i. e., elongated at right angles to the line of the chain)." The opinions of Puppel, Kocher and Tavel, von Lingelsheim, Smith and Brown, Holman and others on this point are not mentioned. The references to the work of Ernst and Trommsdorff are to be found under *Streptococcus mastitidis*, and the impression is given that it is not of assistance as confirmatory evidence in the distinguishing of many hemolytic streptococci. The fact that the authors of the present volume have included only six streptococci, which were hemolytic at the time they were studied, in the 103 cultures used for the illustrations, may explain their conclusion that "as a rule they (the cocci) are elongated in the line of the chain into a lanceolate shape." The typical morphology of streptococci, as is well known, can best be seen in serum mediums or in tissues from the animal body. The important study of D. Taddei (Beitrag zum Studium der Morphologie des Streptococcus, *Centralbl. f. Bakteriol.* **50**:561, 1909) is not referred to in this discussion.

In the review on mediums, there are also many omissions. Under milk medium, there is not any mention of the work of E. Wirth (Zur Kenntnis der Streptokokken, *Centralbl. f. Bakteriol.* **99**:266 and 438 [Aug. 5 and 20] 1926), although this investigator particularly stressed the use of milk as a basic medium; nor is this important article elsewhere referred to, although it contains a very real attempt at classification. A paper by D. D. Todd on "A New Color Medium for the Isolation and Differentiation of Streptococci" (*J. Infect. Dis.* **7**:73, 1910) is also missing. A serious consideration of serum mediums certainly should include the investigation of C. Ten-Broeck on "The Effects of Enzymes in Serum on Carbohydrates and Their Relation to Bacteriologic Technic" (*J. Exper. Med.* **32**:345, 1920). Although Muller's work (1920-1921) on the analysis of the growth requirements of streptococci is given, reference is not made to the importance of phosphates

as studied by H. R. Whitehead (Phosphates and Growth of Streptococci, *Biochem. J.* **20**:1147, 1926), nor to his earlier studies on "Bacterial Nutrition" (*ibid.* **17**:742, 1923; *ibid.* **18**:829, 1924).

The discussion on "Haemolysis," although quite extensive, does not include the important series of studies by K. Ando, N. Ito and their associates (*Kitasato Arch. Exper. Med.* **5**:1, 23 and 30, 1922; *ibid.* **6**:1, 30 and 39, 1923; *ibid.* **7**:29 and 51, 1926). Another perhaps more important oversight is the series of three articles by W. Rother; "Die Beeinflussung des hämolytischen und grünen Wachstum der Streptokokken auf Blutagar durch den Zuckergehalt des Nährbodens" (*Deutsche med. Wchnschr.* **51**:66, 1925); "Ueber die Hämolyse der Streptokokken: II. Mitteilung" (*ibid.*, p. 522); "Ueber die vergrünung des Blutagars durch Streptokokken" ( *ibid.*, p. 1031). In these articles are found fundamental experiments on the factors causing hemolysis and greening by the streptococci. Rother gave, for example, experimental proof that hemolytic streptococci digest hemoglobin, and explained other phenomena on blood agar plates on the basis of the physicochemical movement of the blood pigments.

"The Action of Bile on Streptococci" has received less than one page and covers the review of only one article, although it is an important means of differentiating streptococci into groups. There are, however, a number of references to the use of bile under the discussion of *Enterococcus, Streptococcus* and *Streptococcus faecalis,* and in the differentiation of *Pneumococcus.* The particularly important studies of H. Schönfeld (*Centralbl. f. Bakteriol.* **99**:388, 1926) and K. Meyer and H. Schönfeld (*ibid.*, p. 402), are not referred to, although there is a reference to the third report of this series by K. Meyer on "Hemolytic enterococci" (*ibid.*, p. 416). Among other important articles dealing with this group and omitted from this review are those of: C. J. Oppenheim (*J. Infect. Dis.* **26**:117, 1920), K. Meyer (*Klin. Wchnschr.* **32**:2291 1924), H. Schmitz (*Centralbl. f. Bakteriol.* **96**:277, 1925), S. Went (*Centralbl. f. Bakteriol.* **100**:62, 1926) and N. Kovacs (*Ztschr. f. Immunitätsforsch. u. exper. Therap.* **49**:450, 1926).

In the consideration of enzymes and biologic products of streptococci, no notice is given to the report of W. P. Thompson and F. L. Meleney on "A Comparative Method for Testing the Enzymes of Living Hemolytic Streptococci: I. Lipase" (*J. Exper. Med.* **40**:233, 1924). References to Schottmüller's work and his opinions on "Streptococcic Classification" end with the year 1911, and even such an article as "Ueber die Arverschiedenheit der Streptokokken" (*München. med. Wchnschr.* **71**:1009, 1924) is not mentioned. A short report of W. Zangemeister's on "Temperaturempfindlichkeit der Streptokokken und Fiebertherapie" (*Monatschr. f. Geburtsh. u. Gynäk.* **61**:248, 1923) should also have found a place.

The entire problem of variation is treated much too cavalierly, only three references being given to the extensive studies of J. Morgenroth and his associates. The studies of R. Freund, E. Wirth and several others are not mentioned, and the comprehensive investigations of Kuczynski received only one reference in the bibliography and are not quoted in the text. One, at least, of these reports should have attracted the reviewer's attention, namely, M. H. Kuczynski's "Untersuchungen über Ernährungen, Rassenbildung und Immunität bei Streptokokken" (*Klin. Wchnschr.* **1**:1413, 1922).

There are not any references to the work of E. Phillip, C. Ruge, M. Solis-Cohen, L. Radice, E. Wirth and many more who have attempted the determination of the virulence of streptococci by culturing them directly in blood. This latter group of studies should have been considered in this volume dealing with the streptococcus group in general and particularly with methods of differentiation. Possibly the discussion of virulence will find more consideration in volume IV (to be published), but a reference should certainly have been given here to the report of A. R. Felty and A. L. Bloomfield on "The Relation of Vegetative Activity of Bacteria to Pathogenicity" (*J. Exper. Med.* **40**:703, 1924), which considered problems of invasion, virulence and pathogenicity of streptococci.

I am thus calling attention to the failure on the part of the authors adequateiy to cover the almost endless literature on streptococci, because of the nature of

the publication, and because of its apparent freedom from restraint in respect to space. This freedom is evidenced by the unnecessary repetitions verbatim of whole paragraphs, and the absence of any serious attempt at condensation.

In a consideration of the value of any method of classification, it is indeed difficult to decide on the criteria necessary for any particular purpose. The three essentials for classification of living organisms as given by H. W. Crowe are: 1. Types must be constant. 2. Individuals or types must be recognizable, i. e., "able to be known again." 3. Biologic characters must be diverse enough for differentiation but constant enough for identification. If one takes these criteria and examines them critically, one finds that the chief characteristics for distinguishing the various groups (form and reaction of colonies on his medium) are far from constant. They merely indicate that the individual bacteria, as taken from the immediate environment of the body and still surrounded by the body fluids, develop into a great variety of colony types. He "can distinguish as many as a dozen varieties from one swab from the throat or from one sample of faeces." Of what fundamental importance is this? Two elm trees exactly alike have never been seen, and one could go on dividing them or any other living organism ad infinitum. His B, or "yellow," group, he says, "must be regarded as being in a very fluid state." Characteristics so variable that identification can be made only on the first plate, when there is not any proof that one is dealing with a pure culture or that it is anything more than a growth response to the materials from the body fluids, can hardly be looked on as a stable criterion for the purpose of classification. The acceptance of such a method would practically preclude all experiments on the individual cultures, such as might be desired in detailed biologic tests or in injections in animals. In the example chosen to demonstrate the possibilities of his method, a certain streptococcus, type B, 5(1)(a), had been isolated from the blood of a patient. This is shown in the colored plates (figs. 12 and 13) as subculture colonies, after forty-eight and seventy-two hours' growth. After convalescence, cultures were made from a number of sources in the search for this type. Those from the feces gave a type B, 5(3)(a) (not illustrated) which resembled the colony form of the blood culture strain but differed in fermentation reactions. Cultures from apically infected teeth gave a strain the same as the blood culture strain by the fermentation tests, but with a broader yellow ring about the colony on Crowe's medium (colored plate, fig. 11). The tooth strain was then grown for six "generations" in fresh human defibrinated blood, and it became identical with the blood culture strain. Hence, a change in environment altered the colony appearance, and the actual differentiation from the fecal strain depended on the fermentation of lactose. Crowe's group D, which only blackens the medium, did so with certain strains in mass cultures but not in isolated colonies, while others gave green on primary culture and black on the first subculture. Certain strains of group C (yellow) produced black on subculture. Many other varieties were separated by minor unstable differences.

The second of Crowe's essentials is that the strains shall be identifiable. This is apparently possible only if they come from exactly the same environment and this is the body tissues. Cultures not identified at the time of isolation or those used for experimental work are lost as far as classification by his method is concerned, since it is impossible to determine whether the latter cultures are isolations from an originally mixed colony or not, and, in animal work, whether the culture from the animal is the strain injected or one from the animal itself. The third essential is also only weakly supported by his method, as illustrated in the foregoing comments and by a survey of his tables. Just how Crowe's "notation" differs as a means of classification from J. H. Brown's, which he considers "a form of notation useful perhaps for record keeping but in no sense a classification," I am unable to discover. If one takes, for example, resistance to heat and bile as the important natural character for classification, Crowe's method of "notation" falls to pieces, and if one should add esculin fermentation, as Meyer and others did, I do not know how many changes in his groupings would result.

Classification is of value if the criteria used can be correlated with other facts about the bacteria being studied. These criteria may be different for different purposes and can only be arrived at after analyses of large numbers of organisms. In certain cases, invasive characters may be the dominant consideration or the power of certain bacteria to sensitize the tissues of the body to their proteins, or their growth or autolytic products. Specific toxin production is certainly the important consideration in the etiology of scarlet fever, the fermentation and agglutination tests taking a secondary place; but even production of toxin may not be the whole story in the classification of scarlet fever streptococci. I should believe that resistance to bile may well be a useful criterion for fecal streptococci. In other cases, certain fermentations may be useful in identification and in suggesting natural habitat, but only when tried on a sufficiently large number of strains to determine their value and when used along with other characters. The belief in the specificity of streptococci in producing clinical entities in infected persons is far less fixed than it was only a few years ago.

Why the author chose pneumonia, typhoid fever, leprosy and tuberculosis as examples in which "great advances have been made in the knowledge and treatment of those diseases where the causative germs have been clearly defined," and that these "are all on the way to being cured or prevented" is difficult to see. And why, in the streptococcus group, can the knowledge be said to be "precise" only for the hemolytic streptococci?

One may wonder just how much importance minute classification had in the better understanding of these infections. The knowledge and specific treatment of pneumonia is largely confined to the use of antiserums against the three serologic types, of which type I serum is today usually considered the only effective one. Typhoid fever is prevented by general measures and specific vaccination against the undivided typhoid bacilli. The knowledge, treatment and cure or prevention of leprosy have had little to do with the information about the germ, except in a most general way. The prevention and knowledge of tuberculosis have been advanced through knowledge of stable characters of the germs, but the real progress has been through general measures which help to make the human soil unfavorable. To say that the knowledge of hemolytic streptococci is precise, is speaking broadly. Such accuracy of knowledge as there is certainly has not depended on any type of classification like that suggested.

The Thomsons' own attempts at classification are based on colony differentiation on a medium different from but similar to Crowe's medium, and also on fermentation tests. They used 103 varieties of streptococci obtained from: the British National Collection, twenty-seven; H. Warren Crowe's collection, fifty-one; Edinburgh, one; feces, five; dog with distemper, five; teeth, four; sputum, four; blood in influenza, two; nose, one; urine, one; air, one, and milk, one. The groupings are often quite different from Crowe's groupings, largely because they used a different medium at a different time. One sees under their group A, Crowe's A, C and D groups; under their group B, Crowe's A and C groups; under their group C, Crowe's A, C and D groups; under their group D, Crowe's B, C and D groups. Group E has Crowe's group D alone. It is interesting to note that Crowe's type B, 5(1)(a), (blood), referred to before, is differentiated in group D, 2, by the Thomsons' from Crowe's B, 5(1)(a) (tooth), by the latter's fermentation of lactose, although Crowe said that the two strains from the teeth gave identical sugar reactions and were the same as those from the blood. Here, in the same publication, is a difference of opinion as to the value of the means of classification and, moreover, these were the particular strains used by Crowe to illustrate the advantages of his method. This notation of Crowe's B, 5(1)(a), is found again in Thomsons' group D, 3, as a nonlactose fermenter, and whether it came from the blood or tooth is not stated. It would have been valuable if the numbers of strains falling into the variety headings had been indicated along with their sources so that the relative value of the characteristics given might be determined on some other basis than mere differentiation. The use of Hiss'

serum water as the basic medium for the fermentation tests has little excuse, as even a superficial survey of the literature would have shown that many "high-class" mediums had been used for years.

The use of photography as a means of recording colony forms is undoubtedly a valuable procedure, but until a great many more data indicating the significance of these forms have been collected; until some of the factors involved in the variations have been determined, and until these have been correlated with other facts about the particular streptococci, few will be willing to give colony forms the primary differential standing in a classification of streptococci.

A vast amount of useful material is collected in this volume, but it is to be regretted that so much has been overlooked. At so high a cost ($10), the purchaser should have a reasonably complete presentation of the subject and as full a bibliography, certainly, as the title would suggest. Most of the photographic work is excellent, and this in itself makes this publication a valuable contribution to the literature on streptococci. It would have been better if more time had been devoted to condensation and expansion, and if the difficult problems involved had been more critically analyzed and correlated. Internal evidence suggests that the compilation was rushed.

PHYSIOLOGY AND BIOCHEMISTRY OF BACTERIA. VOLUME I. GROWTH PHASES; COMPOSITION, AND BIOPHYSICAL CHEMISTRY OF BACTERIA AND THEIR ENVIRONMENT; AND ENERGETICS. By R. E. BUCHANAN, PH.D., and ELLIS I. FULMER, PH.D. Price, $7.50. Pp. 546. Baltimore: Williams & Wilkins Company, 1928.

This is apparently the first volume of an ambitiously conceived undertaking on the general physiology of the bacteria. In spite of the preface and introduction, however, it is difficult to grasp the full plan or intent of the work. Perhaps a later volume will demonstrate the justification; but this first volume leaves one definitely of the opinion that the physiology and biochemistry of bacteria cannot be written now: the subject is too insufficiently advanced.

Chapter 1 is introductory. Chapter 2 is concerned with the quantitative analysis of growth phases and growth rates. Although interesting, it is patently largely imaginative. Chapter 3, on the chemical composition of micro-organisms, includes extensive compilations from the literature. The material, however, comes principally from data of "ancient" and uncertain value. Chapter 4, on the physico-chemical characteristics of micro-organisms and their environments, includes half the book. And, though it includes interesting summaries of the elementary concepts in physics and chemistry, the bacteriologic applications are notable by their absence. Then, too, much of it has been done more comprehensively and better elsewhere—the excellent monographs by Mansfield Clark, Michaelis, Freundlich, Burton and other investigators. Why is there a need for reiteration of these subjects in abbreviated forms in an expensive book on bacteria? Chapter 5, on energetics, is also largely academic and not directly concerned with the bacteria. The appearance of the review by Baas-Becking and Parks in 1927—too late to receive more than a footnote in this volume of 1928!—provides a striking illustration of the impracticability of a large treatise on general physiology.

Favorable comments may be made concerning this interesting volume, but the reviewer is of the opinion that the zeal of the authors exceeded their wisdom. General physiology is the field for monographs or for books built on collective contributions.

# Books Received

CHEMOTHERAPEUTIC RESEARCHES ON CANCER WITH ESPECIAL REFERENCE TO THE LEAD AND SULPHUR GROUPS. By A. T. Todd and others. Paper. Price, 2/6. Pp. 127. Bristol: J. W. Arrowsmith, Ltd., 1928.

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The first edition of this book appeared in 1920, and the second in 1922, and now after three reprints of the second edition comes the third, entirely revised and with much new material. The difficult task of revision has been carried out in masterly fashion to meet the present requirements of workers in the biologic sciences.

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